DIETARY FAT EFFECTS ON RUMEN FERMENTATION MILK PRODUCTION AND REPRODUCTION OF DAIRY CATTLE, AND ECONOMIC IMPLICATIONS FOR DAIRY PRODUCTION

By

AMEY L. ADAMS

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KEY TO ABBREVIATIONS

AH- alfalfa hay
BCS- body condition score
bST- bovine somatotropin
C:P- cholesterol to protein ratio
CaLCFA- calcium soaps of long chain fatty acids
CaPF- calcium soap of poultry fat
CL- corpus luteum
CO- corn oil
CS- corn silage
DIM- days in milk
DIP- rumen degradeable intake protein
DMI- dry matter intake
DNS- defined nutrient solution
EOF- erythrocyte osmotic fragility
ES- energy status
FCM- fat corrected milk
FFA- free fatty acids
FO- Menhaden fish oil
GnRH- gonadotropin releasing hormone
HDL- high density lipoprotein
HOC- high oil corn
HOM- hominy
LDL- low density lipoprotein
LH- luteinizing hormone
N:P- nitrogen and phosphorus
NDF- neutral detergent fiber
NEFA- non esterified fatty acids
NEL- net energy for lactation
NES- negative energy status
NSC- non structural carbohydrate
P_4- progesterone
PF- poultry fat
$PGF_{2\alpha}$- prostaglandin $F_{2\alpha}$
PGFM- prostaglandin F metabolite
PUFA- polyunsaturated fatty acids
TAG- triacylglyceride

TAI- timed artificial insemination
TALL- tallow
TFA *trans* fatty acids
TRH thyroid releasing hormone
UIP undegraded intake protein
VLDL- very low density lipoprotein
WCS whole cottonseed

Abstract of Dissertation Presented to the Graduate School
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DIETARY FAT EFFECTS ON RUMEN FERMENTATION
MILK PRODUCTION, AND REPRODUCTION OF DAIRY CATTLE
AND ECONOMICS IMPLICATIONS FOR DAIRY PRODUCTION

By

Amey L. Adams

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The purpose of the current investigations was to examine the effects of supplemental dietary fat on interactions with rumen microbes and subsequent fermentation products *in vitro*, and milk production and reproduction *in vivo* of early lactation dairy cows. The *in vivo* experiment also included recombinant bovine somatotropin (bST) at a dose of 208 mg/14 days to study its potential use to stimulate reproductive endocrine responses and improve pregnancy rate early postpartum.

In vitro experiments utilized six fat sources: whole cottonseed (WCS), tallow, Menhaden fish oil, poultry fat, calcium soap of poultry fat, and corn oil. Fat sources were mixed with hominy, corn silage, or alfalfa hay before components were mixed and incubated with rumen bacteria for 0, 12, 24, 48 and 96 h. Neutral detergent fiber

remaining in samples did not differ between treatments, indicating that dietary fat in this experiment did not inhibit fiber digestibility

In vivo experiment utilized WCS and bST in a 2x2 factorial design Treatments were 0% WCS, 0 bST (T0), 15% of diet dry matter (DM) WCS 0 bST (T1) 0% WCS 208 mg/14 d bST (T2), and 15%WCS plus 208 mg/14 d bST Primi- and multiparous cows (n=186) were assigned randomly to treatments Milk production was increased by WCS (35 09 vs 34 46 kg; p<0 001), as was milk fat percentage (3 45 vs 3 15% p<0 0001) Whole cottonseed increased plasma high density lipoprotein (HDL) cholesterol (p<0 001) and progesterone (P, p<0 001) Plasma HDL was also increased by bST (p<0 001) First lactation animals which calved during the warm months of the trial had 40% greater pregnancy rates to two timed artificial inseminations when receiving bST (p=0 0216)

Finally, a linear program was constructed of an existing dairy farm to model the potential financial impact of research efforts in nutrition and reproduction on the farm enterprise Ration balancing and whole farm nitrogen and phosphorus balancing were included as elements of the program The model selected WCS at the maximum allowed level to meet energy and protein requirements The model also selected bST as an alternative technology to increase animal productivity

CHAPTER 1
INTRODUCTION

The dairy industry in the US faces many challenges, not the least of which is maintaining profits in the face of variable feed prices, environmental regulations, and decreasing federal support for agriculture. Previously, drought in the Corn Belt had driven feed commodity prices to their highest point in 30 years. The 1996 Federal Agricultural Improvement and Reform Act (H.R. 2854) will phase out most crop subsidies over seven years. Crops affected by this change in policy will include corn and soybeans, which have been staple commodities for most livestock feeds. This legislation will also phase out the Dairy Support Price program over four years.

In addition, environmental regulation of livestock waste disposal has become a major public concern, and much of the focus for this issue in Florida has been on dairies. Dairymen are now required to develop manure disposal systems in order to comply with Department of Environmental Protection water quality standards (Twachtmann 1990). This has led to considerable research efforts which emphasize nitrogen (N) recycling and address such issues as maximum carrying capacity and nutrient uptake by crops (Van Horn, 1992).

The approval of recombinant bovine somatotropin for use in dairy cattle to increase milk production has led to its widespread adoption by dairy farmers.

Somatotropin is a naturally occurring peptide hormone which is synthesized by the pituitary and has activities in numerous tissues. Lactating dairy cattle differ in the quantity of somatotropin they synthesize. Use of exogenous somatotropin increases milk production, dry matter intake, and the efficiency of conversion of nutrients to milk, increasing animal productivity (Bauman, 1992). However, because it increases the partitioning of feed nutrients to the mammary gland, it may prolong the period of negative energy status and lengthen the calving interval.

Lactating dairy cows require high quality, high energy and protein feeds in order to produce to their potential. Feed costs are thus the most significant expense to the dairy, traditionally comprising 50 to 70% of the gross income from milk (Buchman, personal communications). In 1995, this proportion fell to 40%, but climbed again to 48.5% of gross receipts from milk in 1996 (Hockema et al., 1998). It has become increasingly apparent in recent years that current nutrient definitions and requirements are inadequate, and require reexamination in order to improve animal health and efficiency of feed conversion to milk (Hall, personal communications). Dietary energy requirements to support copious milk production in the early postpartum period are at their greatest when dry matter intake is generally reduced. As a result, cows mobilize body stores of fat and protein. This situation, termed negative energy status, may compromise reproductive function and lead to metabolic disease.

Dietary fat has typically been added to dairy rations to compensate for decreased dry matter intake during the early postpartum period. Supplemental fat was thought to reduce negative energy status, thus preventing body condition loss and its associated

problems, and increase milk production. This has not always been the case, however, as animals in various trials have responded differently to dietary fat. More recently, evidence has emerged indicating that fat may improve reproductive status in cows apart from energy status through increasing plasma cholesterol and altering concentrations of circulating reproductive hormones.

Reproduction has become a major issue of concern to dairymen in Florida. Artificial insemination (AI) is commonly part of reproductive management. High peak production shortly after calving, combined with extended periods of heat stress contributes to low conception rates to AI on Florida dairies, and calving intervals are longer than in cooler areas of the country (Badinga et al.,1985). Cows typically return to AI service four to five times, and cows which do not conceive until mid- to late lactation do not produce to their lifetime potential. Reproductive failure is a common reason for involuntary culling, and, because the replacement rate on many Florida dairies is 35 to 40% (Webb, 1994), poor conception rates mean fewer replacement heifers raised on farm for genetic advancement of the herd and increased expenditure for replacements.

Considerable research efforts have been devoted to resolving these issues. In the area of reproduction, for example, many technologies have been developed, including nutritional supplements and hormonal treatments, while other approaches are still being studied. Unfortunately, although some of these technologies are indeed effective, little attention has been given to their financial impact on the farm.

The objective of the current research is to study the effects of dietary fat on *in vitro* fermentation of dietary fiber and interactions between fat source and rumen bacteria,

and effects on animal production and reproduction of early lactation Holstein cows managed under a timed artificial insemination protocol In addition, exogenous recombinant bovine somatotropin (bST) was incorporated into the whole animal trial to study the potential interaction of bST and diet on ovarian follicular dynamics, blood metabolites, reproductive hormones, and pregnancy rates

The fat source chosen for the whole animal experiments was whole cottonseed, a commonly used oilseed in dairy rations in the southeastern U S Whole cottonseed is a source of both fat (20%) and protein (23%), and is generally an inexpensive commodity Cottonseed oil is also high in polyunsaturated fatty acids, which may provide the added benefit of preventing early embryo loss in some cattle However, cottonseed also contains an antiquality factor, gossypol which has been shown to have negative effects on reproduction in nonruminant animals, while its effect on ruminant females, if any remains to be determined.

Finally, a model was constructed using linear programming to examine the effects of nutritional and reproduction research on the whole dairy operation, using data gathered from an existing large scale dairy in North Central Florida. The model incorporates ration formulation in order to examine the value of nutritional supplements within the context of meeting animal nutritional requirements while optimizing farm income. Whole farm N and P balancing was also incorporated as part of the matrix to examine the impact of nutrient management regulations on farm production and income

CHAPTER 2
LITERATURE REVIEW

Dietary Fat for Ruminants

Meeting the nutritional requirements of high producing dairy cattle is a challenge of increasing difficulty and importance for the dairy industry. Within six weeks of calving cows reach peak milk production for that cycle, while preparing to return to normal reproductive function. However, feed intake during these first weeks of lactation is generally depressed, and consumption will not peak until approximately 12 weeks postpartum. As a result, cows must mobilize body stores of fat and protein to maintain milk production, which has metabolic priority over reproduction. This state has been termed negative energy status (NES) (Staples et al., 1991).

If NES is severe, milk production declines and loss of body condition is marked. These problems are further aggravated in sub-tropical and tropical climates by heat stress, which further depresses feed intake and milk yield, and may be both directly and indirectly responsible for poor conception rates.

In order to compensate for depressed feed intake by early lactation cows, the solution has been to increase calorie density of the diet. This may be achieved in two ways. The first method is to increase the readily fermentable carbohydrates in the diet by

increasing the amount of grain fed and, hence, reducing the amount of forage. This, however, may lead to metabolic disorders such as lactic acidosis.

An alternative to increasing carbohydrates in the diet is to add fat. Fat has approximately 2.25 times the calories of carbohydrate per gram, and can be effectively added to the ration maintaining forage content. Supplemental fat was expected to increase milk production by providing more energy and sparing glucose for milk synthesis, and to reduce NES in early lactation. Also, supplemental dietary fat was thought to improve reproductive function through its effects on ES and by increasing hepatic synthesis of cholesterol, the precursor of progesterone (P_4).

Responses to fat supplementation have been highly variable, however. Milk yield in response to fat feeding has either increased (Clapperton and Steele, 1983, Jenkins and Jenny, 1989, Moallem et al., 1997, Pires et al., 1997) decreased (Horner et al., 1986), or remained unaffected (Hawkins et al., 1985, Lough et al., 1988, Mohammed et al., 1988, Bertrand et al., 1998, Jenkins, 1998). Reports of effects on ES in early postpartum cows also have been conflicting (Villa-Godoy et al., 1988, Butler and Smith, 1989, Villa-Godoy et al., 1990, Lucy et al., 1991a, Braun and Butler, 1998).

Komaragiri et al. (1998) noted that cows fed diets containing 3% added fat as Megalac® (a Ca soap of palm oil fatty acids) tended to mobilize less body fat (37 vs. 66 kg) compared to controls, but mobilized more body protein stores (19.5 vs. 4.8 kg).

After decades of research, some of the variability in responses to supplemental dietary fat have been explained, but much still remains to be elaborated. This variability

has been attributed to the source of fat used and the complex interactions between fat, other feed ingredients, the rumen micro environment, and animal metabolism

Fat Source and the Rumen Environment

Negative effects of fat on the rumen microbial population have been noted by several authors. Source of lipid and its composition influence rumen microbial populations directly and indirectly through interactions with other feed ingredients

Rumen fermentation patterns in vitro were altered substantially by the presence of long-chain fatty acids less than 18 carbons in length and by unsaturated 18-carbon fatty acids, but not by stearic acid (18 0), an 18-carbon, saturated fatty acid (Chalupa et al., 1984). Long-chain fatty acids with high melting points such as 18 0 from tallow and calcium soaps of long-chain fatty acids (CaLCFA) decreased the acetate propionate ratio less than fatty acids of equal length but with lower melting points such as oleic acid (18 1ω9) *in vivo* (Chalupa et al., 1986) Acetate is the major precursor for fat synthesis by the mammary gland.

Fatty acids may be toxic to certain species of rumen microbes In some instances this may be beneficial Dietary fat is thought to improve feed efficiency and bacterial nitrogen production through negative effects on rumen protozoa, which are known to prey on bacteria. The reduction in acetate propionate ratios frequently observed when fat, particularly unsaturated fatty acids, is fed has been attributed to detrimental effects on fiber digestion due either to direct toxic effects on cellulolytic bacteria or preventing solid adherent bacteria from attaching to fibers (Jenkins, 1993a)

Devendra and Lewis (1974) suggested four possible mechanisms to account for the observed effects of supplemental fat on the rumen microbial population. First, physical coating of the fiber with fat may prevent microbial colonization. Second, modification of the microbial population, such as reduction in numbers of cellulolytic bacteria, may result from direct toxic effects of fat on these species. Third, surface active effects of fatty acids on cell membranes may inhibit microbial activity. Lastly, fatty acids may form insoluble soaps with Ca and Mg, reducing the availability of cations required by bacteria.

Jenkins (1993a) suggested that the mechanism of lipid interference with fermentation depends on a number of interacting factors such as uptake of lipids into the microbial cell membrane, ability of the lipid to disrupt the membrane, attachment of the microbial cell to plant surfaces, and expression and activity of enzymes. Forage quality and amount of surface area of the particles and structural modification (biohydrogenation) of the lipid molecule may all interfere with activity of bacterial enzymes.

Fat source

Rumen microorganisms do not utilize fatty acids for energy. However, triglycerides are hydrolyzed in the rumen to free fatty acids and glycerol. Glycerol is fermented by rumen bacteria to produce propionate. The rate and extent of lypolysis appears to depend on the source of fat (Jenkins, 1994).

Reddy et al. (1994) studied the extent of lipolysis of soy oil, raw soybeans, and roasted soybeans in vitro at different incubation times. Release of free fatty acids from oil was most rapid, reaching maximum levels after four hours incubation with rumen fluid.

Raw and roasted whole soybeans released the most fatty acids after 6 and 12 hours, respectively

Fatty acids may be taken up by rumen microbes for use in membrane synthesis Bauchart et al (1990) noted that free fatty acid (FFA) content of both solid-adherent and liquid-associated bacteria increased 150% in rumen digesta obtained from dairy cows fed supplemental fat compared to samples from unsupplemented cows. In addition, using transmission electron microscopy lipid droplets were observed in the cytosol of both solid-adherent and liquid-associated bacteria from fat supplemented cows.

Dietary fat, both FFA and triglycerides, have been shown to adsorb onto the surface of feed particles in the rumen by hydrophobic interactions, and so are generally believed not to be free floating in rumen fluid (Sukhija and Palmquist, 1990) Bauchart et al (1990) noted that total lipids of solid adherent bacteria from fat supplemented cows was 1 7 to 2.2 times greater than in liquid associated bacteria from the same animals

In studies with rumen microorganisms (Jenkins, 1993a), substituting the fatty acid carboxyl group with another functional group reduced its negative effects. This suggests that a free carboxyl group is necessary for disruption of normal microbial cell function. This would help to explain why triglycerides and other bound forms of fatty acids such as calcium soaps and amides have fewer effects on rumen fermentation patterns.

To prevent negative effects of fat on rumen microorganisms, and to protect fatty acids from alteration in the rumen, several methods have been used to create fat supplements resistant to bacterial activity These products include calcium soaps, fatty

acid-amino acid conjugates or amides formaldehyde and/or heat treatment of oil seeds and fish oil, and coating fats with serum or gelatin.

Early research on oilseeds hypothesized that whole seeds resisted rumen degradation because bacteria would not be able to penetrate the hard seed coat. However feeding trials revealed that little or no whole cottonseed passed intact to the abomasum of cattle. Rather, most of the seed coats were broken by mastication, and subsequently much of the lipid was broken down in the rumen (Jenkins 1993a)

Calcium soaps of long-chain fatty acids (CaLCFA) have received greatest attention over the past decade, and have been the subject of numerous trials. Palmquist and Jenkins (1980) theorized that fatty acids bind to free divalent cations in the rumen, primarily calcium and magnesium, forming insoluble soaps at the near neutral pH of rumen fluid. These soaps would then dissociate in the acid environment of the abomasum, and would be absorbed in the duodenum as FFA and cations. Based on this theory, Sukhija and Palmquist (1990) developed a calcium soap of palm oil fatty acids which had a pKa greater than 4.6, meaning that half the fatty acids would dissociate from the cation at that pH. Other soaps were also tested including calcium soaps of tallow, rapeseed oil, and soy oil. Unfortunately, the products of oils high in polyunsaturated fatty acids (PUFA) have a sticky consistency, making them more difficult to incorporate into feed, and had pKd of 5.6 or greater, depending on the degree of unsaturation of the product. Maximum dissociation of these soaps occurred at pH 5.0, with minimum dissociation at 6.5. Calcium soaps of palm oil were judged to be stable at pH 5.5.

Fish oil and fish meal also have been tested as potential sources of energy for dairy cattle, but the results have been somewhat conflicting. Fish products are high in very long-chain PUFA, which have been variously reported to having no effect (Spain et al., 1995) or detrimental effects on rumen microbes (Palmquist and Kinsey, 1994). Hoover et al. (1989) reported that detrimental effects of high oil fish meal were noted only when continuous cultures were maintained at pH 6.2, resulting in decreased acetate:propionate ratio, decreased production of microbial protein, and decreased protein and dry matter (DM) digested. When pH was permitted to decrease to 6.0 or below, acetate:propionate ratio and neutral detergent fiber (NDF) digestion were greater in fish meal treated cultures than cultures receiving soybean meal (Hoover et al., 1989).

Biohydrogenation

A typical response of rumen microbes to free unsaturated fatty acids is to hydrogenate the double bonds. There are two theories regarding biohydrogenation. It may be in order to render them less toxic, or unsaturated fatty acids may serve as electron acceptors (or hydrogen sinks) in anaerobic respiration. When fat content of the diet is low, hydrogenation is virtually complete, yielding saturated fatty acids. However, as fat content increases, the capacity of the rumen microorganisms to hydrogenate fat may be overwhelmed, resulting in a variety of *trans* isomers.

It is generally recognized that biohydrogenation is carried out by rumen bacteria, rather than protozoa. In order to carry out hydrogenation, bacteria apparently require a free carboxyl group. Thus, only nonesterified or FFA may be refluxed in this manner.

Linoleic (18:2ω6) and linolenic acids (18:3ω3), the most common unsaturated fatty acids in plant material, generally are reduced to 18:0

Another product resulting from partial hydrogenation of these acids are *trans* fatty acids, the most common of which is *trans*-octadec-11-enoic acid, commonly referred to as vaccenic acid Vaccenic acid may comprise 5 to 10% of ruminant adipose tissue (Mackie et al , 1991, Jenkins, 1993a)

Most lipids in animal feed occur in esterified form as triglycerides or phospholipids. Hydrolysis of these ester bonds is carried out by extracellular lipases and esterases, produced by bacteria such as *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* Bacterial lipase, unlike mammalian lipase, completely hydrolyzes triglycerides to FFA and glycerol, with virtually no accumulation of mono- or diglycerides (Jenkins, 1993a)

Bacteria responsible for biohydrogenation fall into two distinct groups The first of these groups consists of species capable of hydrogenating 18:2ω6 and 18:3ω3 to vaccenic acid, but cannot reduce vaccenic to 18:0 The second group of species is capable of hydrogenating many 18 carbon fatty acids including oleic acid (18:1ω9) 18:2ω6, and vaccenic acids to 18:0 This second group reduces 18:3ω3 to *cis*- and *trans*-octadec 15-enoic acids which are not further hydrogenated by rumen bacteria. The first group contains most of the isolates including one strain of *Ruminococcus albus* (F2/6), two strains of *Eubacterium*, and several strains of *Butyrivibrio*. Only three species have been identified as belonging to the second group two strains of *Fusocillus* and no unidentified Gram-negative rod (Mackie et al , 1991)

It has been hypothesized that one of the primary functions of hydrogenation is the disposal of excess reducing potential by bacteria. However, there is currently no evidence to support this theory. The process of biohydrogenation appears to occur through a completely separate pathway using ferredoxin or a-tocopherolquinol as electron donors. The disposal of reducing power, on the other hand, occurs mainly through the reduction of CO_2 to CH_4 (Mackie et al., 1991).

Because a separate pathway exists for the hydrogenation of unsaturated fatty acids, it is likely that biohydrogenation serves some protective role. Fatty acids have been noted to have similar cytotoxic effects in both bacteria and eucaryotic cells by disrupting cell membrane function. Fatty acids in pure cultures adsorb to cell membranes and inhibit growth and metabolism (Jenkins, 1993a).

Wu and Palmquist (1991) noted that biohydrogenation of unsaturated fatty acids from animal-vegetable blended fat reached 71%. Linoleic acid, an essential fatty acid precursor of prostaglandins, was biohydrogenated by as much as 89% in some experiments performed in vitro. Accumulation of *trans* 18:1 and 18:0 after incubation was greater with animal-vegetable blend supplements than when CaLCFA were the substrate.

Similar results were reported in an in vivo trial (Wu et al., 1991), 87% of unsaturated fatty acids in animal vegetable blends were biohydrogenated. Biohydrogenation of 18:1ω9 ranged from 64.8 to 67.8% in diets containing 3 or 6% animal vegetable blend fat. Hydrogenation of more unsaturated fatty acids in these diets were higher (18:3ω3: 82.7 to 84.8%, 18:2ω6, 75.3 to 78.4%).

In an experiment to increase the amount of 18 1ω9 in the adipose tissue of steers high oleate sunflower seed was fed either with or without encapsulation to protect the seeds from microbial attack (Ekaren et al., 1992). Samples taken from the proximal duodenum via cannula had greater concentrations of 18 0 for both seed treatments compared to controls. Levels of 18 0 were higher for unprotected seed than for the encapsulated seed treatment. Although 18 0 concentration was low in all diets (3% or less), it was the most abundant fatty acid in digesta and feces for all treatments, reflecting biohydrogenation of 18 1ω9 in the rumen. Concentration of 18 2ω6 was unaffected by treatment, although concentrations of this fatty acid ranged from 35.2 to 52.4% in sunflower and control diets. It should be noted, however, that standard errors in this study were very large, so that differences could not be detected for most treatments (mean 18 2ω6 in digesta, 144.4 μg/g, SE 180.8 μg/g; mean 18 2ω6 in feces, 348.1 μg/g, SE 590.8 μg/g).

An additional study attempted to increase concentration of 18 1ω9 in milk fat by feeding high oleic sunflower oil at 3.7% of the diet to lactating cows (Kalscheur et al., 1997b). High oleic sunflower oil contains 78% 18 1ω9 and 11% 18 2ω6. Flow of 18:0 and *trans* 18 1ω9 to the duodenum were increased when either high oleic or high linoleic sunflower oil was fed. Although flow of *trans* 18:1 was lower in the high oleic sunflower oil treatment, flows of 18:0 were similar for both oil treatments and higher than in control animals not receiving supplemental fat. Milk fat of cows receiving high oleic sunflower oil had high concentrations of both *cis* and *trans* 18:1

In sheep, amount of 18:2ω6 reaching the duodenum was 20 g per 100 g free 18:2ω6 administered compared to 71 g 18:2ω6 per 100 g administered as linoleoyl methionine (Fotouhi and Jenkins, 1994)

Palmquist and Kinsey (1994) found that concentrations of 18:2ω6 and 18:3ω3 in animal vegetable blended fat decreased rapidly in vitro from a total of 15% to 5% during the first 6 h of incubation. Concentrations of 18:0 increased from 7 to 20%, while *trans* 18:1 increased from 0.8 to 7% in the same cultures.

The twenty and twenty-two carbon ω-3 PUFA of fish oil resisted biohydrogenation when incubated in rumen fluid cultures in vitro, although triglycerides were rapidly hydrolyzed (Ashes et al., 1992). After 24 hours incubation, the concentration of eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids were virtually unchanged (13% 20:5ω3 after 24 h incubation vs. 13.3% in fish oil). Eighteen carbon mono- and polyunsaturated chains, principally 18:1ω9 and 18:2ω6, contained in the fish oil were hydrogenated, however.

Fiber digestion

Palmquist and Jenkins (1980) suggested that the reason for variable responses in milk yield to fat supplementation was due to decreased fiber digestion in the rumen. Since cellulolysis is carried out mainly by solid-adherent bacteria, it is possible that physical coating of forage with fat, as suggested by Devendra and Lewis (1974), prevents attachment of these bacteria directly to cellulose containing fibers. However, subsequent research has failed to show a decrease in numbers of solid-adherent bacteria attaching to fiber particles. It is possible that lipid coating of fibers somehow interferes with

extracellular cellulases, perhaps through preventing binding of these enzymes with their substrate (Jenkins, 1993a)

Jenkins and Jenny (1989) reported decreased acid detergent fiber (ADF) digestibility in cows fed yellow grease or hydrolyzed fat compared to control animals Similarly, Smith et al (1993) observed decreases in both ADF and NDF digestibilities in cows fed either whole cottonseed (WCS) or tallow or the two fats combined Negative effects on fiber digestion were more marked for diets containing tallow alone compared to WCS, and effects were more negative for diets where corn silage was the sole forage than when alfalfa hay was added to the diet Adams (1993) also observed decreased fiber digestibility in cows supplemented with fat when the forage was corn silage, but NDF digestibility improved with the addition of alfalfa hay to diets with supplemental fat. Neutral detergent fiber digestibility was higher for cows consuming raw WCS compared to roasted WCS or no WCS diets in a study by Fires et al (1997)

In contrast, Reddy et al (1993) noted reduced NDF and ADF digestibility in vitro when ground alfalfa hay was incubated with soy oil or whole soybeans However, digestibility of fiber fractions was higher for samples containing whole seeds versus those in which free oil was added Decreases in rumen organic matter (OM) digestibility in vivo were mainly attributed to decreased fiber digestion in a study by Ferlay and Doreau (1992) when rapeseed oil was administered either in a single dose or as a continuous infusion into the rumens of midlactation cows

After reviewing available data on the effects of fat on fiber digestibility and subsequent milk production response, Jenkins (1993b) stated that quantity of unsaturated

fatty acids was a better predictor of milk response than total added fat. Specifically, the amount of unsaturated fatty acid per unit of ration ADF was the best predictor of milk response to supplemental fat. He suggested that the optimum ratio of unsaturated fat:ADF was 0.06.

Post-Ruminal Digestion and Metabolism

Supplemental dietary fat has resulted in a variety of responses in the whole animal. Changes in body weight (BW) and composition, composition and metabolism of adipose tissue, production and composition of milk, nutrient digestion and absorption, glucose metabolism, plasma hormones, and ovarian follicular dynamics have been reported, but experimental results from many trials are conflicting.

Fat feeding to early lactation cows was expected to decrease weight loss generally observed during the first 6 weeks of lactation, when milk production peaks but feed intake is frequently below that necessary to meet requirements. A number of methods have been used to estimate the effects of dietary fat on metabolism of early lactation cows, including measurements of BW, body condition score (BCS), and calculation of ES.

Body condition scoring is a system developed to express an animal's relative fatness or thinness. It is based on a scale of one to five for dairy cattle (1 to 10 for beef cattle), with one representing emaciated animals and five to identify obese animals. The optimum for dairy cattle is between 3.0 and 3.5, although variations are expected during the course of the lactation cycle.

This system is arguably more precise than BW measurements, as BW fluctuates considerably depending on the amount of rumen fill, while BCS is based on the amount of

flesh covering the back, pelvic region and tail head area, which vary less over short periods Also a single individual is generally consistent in scoring animals

Energy status is calculated based on the amount of energy in feed consumed less the output of energy in milk, less energy required for maintenance Because of the potential errors involved with all of these measures it is generally recommended that at least two methods be used in conjunction with one another

Negative ES can result in impaired reproduction, animals become anestrous, or estrus behavior is suppressed, and conception rates decline (Staples et al., 1991) In most experiments which studied the effects of fat on metabolism in lactating cows, fat supplementation did not affect BW or BCS gain after peak lactation Instead, fat tended to increase BW loss during early lactation, even though it increased calculated ES (Chilliard, 1993)

Many studies have noted decreased feed consumption when fat is added to the diet In most cases the decrease in intake was such that animals receiving fat consumed approximately the same calories as control cows receiving no fat (Chilliard, 1993) This response, however often depends on the type and amount of fat fed, since amounts of fat exceeding 6% of total ration DM appear to cause greater depression in intake (Schauff et al , 1992) Pantoja et al (1996) reported linear decreases in dry matter intake (DMI) with increasing unsaturation of supplemental fat As discussed earlier, low melting point fats high in unsaturated fatty acids also may cause a depression in feed consumption due to negative effects on ruminal digestion of other feed ingredients principally fiber (Chalupa et al , 1984)

Post ruminal lipid infusion studies

One method used to separate the effects of fat on the rumen environment from subsequent animal responses has been to infuse fat directly into the abomasum or proximal duodenum of cattle. Infusion studies have indicated that many effects of fat on intake, digestion and absorption of lipids and other nutrients as well as their effect on animal metabolism and performance, are independent of their effects on the rumen environment.

Christensen et al. (1994) infused four types of fat into the abomasum of cows to examine post-ruminal effects on nutrient digestibility, metabolism and milk production. The four fat sources were either mostly saturated fatty acids or fatty acids from canola, soybeans, or sunflower seeds. Control animals were infused with most solubles. They found that fatty acids decreased DMI compared to controls, and that cows infused with saturated fatty acids consumed more protein and digestible energy than cows infused with the more unsaturated fatty acids, indicating that unsaturated acids also have effects beyond the rumen. Fatty acids from soybeans also decreased DMI compared to saturated fatty acids.

Abomasal infusion of canola or high oleate sunflower oil at different levels into lactating dairy cows decreased DMI and resulted in linear increases in triglyceride rich lipoproteins in plasma, which then decreased when amount of oil infused was decreased (LaCount et al., 1994). Plasma cholesterol also increased and NEFA tended to increase with oil infusion in this trial.

Infusions of fat high in *cis* 18:1ω9 (64% high oleic sunflower oil plus 32% cocoa butter) were compared to infusions of fat high in *trans* 18:1 (90% partially hydrogenated

soybean oil plus 10% high linoleic safflower oil) and no infusion controls for their effects on energy and N metabolism in midlactation cows (Ronne et al., 1996). Feed intake was decreased for fat-infused cows compared to controls. Fat infusion reduced fecal and urinary N output, but did not affect overall N balance as intake of N was reduced by fat treatment. Energy intake was not different among treatments, but energy output in milk was higher overall for fat-infused cows. Energy losses in feces, urine and gas were less for cows infused with fat compared with controls.

Rumen inert fat studies

As mentioned earlier, numerous studies have been conducted on the effect of protected or rumen inert fats on feed intake, digestibility, absorption, and metabolism.

Schauff et al. (1992) compared the effects of extruded soybeans and CaLCFA, separately or in combination, on DMI and digestibility of feed components, including fatty acids. Dry matter and energy intake increased when extruded soybeans were fed alone, but decreased when CaLCFA were added. Apparent digestibility of DM, OM, ADF, soluble residues, total 18 carbon fatty acids, and total fatty acids were decreased by supplemental dietary fat, although digestion of hemicellulose increased when fat was supplemented to the diet. The largest decrease in intake and digestibility occurred with the highest level of fat supplementation (16% extruded soybeans plus 6% CaLCFA).

Differences in total tract digestibility of fatty acids were noted by Klusmeyer and Clark (1991) when CaLCFA were fed to cows in combination with different protein sources. In contrast with the study by Schauff et al., (1992), digestibility of total 18-carbon fatty acids were not affected by feeding of CaLCFA in this trial. In fact,

digestibility of laurie (12·0), myristic (14·0), palmitoleic (16 1ω7), 18 0 18 1ω9 and 18 2ω6 increased by addition of CaLCFA to the diet.

Chouinard et al (1998) noted that ADF digestibility was lowest among cows fed Ca soaps of soybean oil (high in 18 2ω6) compared with similar CaLCFA of canola (high in 18 1ω9) and linseed oil (high in 18 3ω3) However this trend was not noted for NDF digestion.

Schauff and Clark (1989) recommended that rumen inert fats such as CaLCFA or prilled fats not exceed 3 to 4% of diet DM Apparent total tract digestibility of DM OM ADF, NDF and crude protein (CP) were not significantly altered when either of these supplements were fed to lactating dairy cows.

It has been suggested that *trans* fatty acids are absorbed less efficiently in the lower digestive tract. This may be a partial explanation for the decreased digestibility of fat in total tract studies, and incomplete utilization of the energy fat is expected to provide to the animal (Jenkins 1993a) Also, conversion of PUFA to monounsaturates or *trans* unsaturated fatty acids may deprive the animal of essential fatty acids. It should be noted, however that measures of apparent total tract digestibility of fatty acids are complicated by the excretion of endogenous fatty acids from the intestinal epithelium and bile.

Supplemental fat and adipose tissue metabolism

In ruminant animals the major site of de novo fat synthesis is adipose tissue, rather than the liver (Chilliard, 1993) Fat is synthesized principally from acetate absorbed through the rumen wall and transported via the blood Fat in the diet has been shown to suppress de novo fat synthesis by adipose tissue. There are two possible mechanisms

involved One is the reduction of available acetate coming from the rumen due to changes in fermentation patterns The other is via feedback inhibition on adipose synthetic enzymes by increased lipids in the blood.

In most mammals, lipids are transported from the digestive tract to the liver in the form of chylomicrons. In humans, most of these particles are metabolized by the liver However, in ruminants lipoprotein lipase associated with adipose tissue is very active in cleaving the triglycerides in chylomicrons and low density lipoproteins into FFA, which are then taken up by adipocytes (Chilliard, 1993)

Fatty acid composition of ruminant adipose tissue can be manipulated through use of supplemental dietary fat. Jenkins et al. (1994) showed that soybean oil added to the diet of sheep changed the proportions of several fatty acids in subcutaneous fat. Levels of 18:2ω6 and *trans* 18:1 increased, while anteiso 17:0, a branched chain fatty acid produced by ruminant bacteria, and 18:0 decreased Concentration of arachidonic acid (20:4ω6), a major precursor of prostaglandins and a component of an important second messenger system in cell metabolism, was nearly 50% lower in plasma membranes of sheep fed soybean oil compared to control sheep

Interestingly Jenkins et al (1994) noted that in vivo fatty acid synthesis from acetate was not affected by soybean oil treatment, and that lypolysis tended to be higher in sheep receiving soybean oil

Fish oil fed to cattle and sheep did not increase the levels of 20:5ω3 and 22:6ω3 fatty acids in adipose tissue However, the proportion of these fatty acids in phospholipids of muscle tissue increased 3 to 4 fold in both species receiving supplemental fish oil In

sheep, proportion of 18 1ω9 declined, but no consistent change was noted for 20 4ω6 among animals receiving fish oil (Ashes et al 1992)

Metabolic hormone responses to supplemental fat

In order to understand the effects of dietary fat on ruminant metabolism, several studies have been conducted to examine hormonal responses to supplemental fat. Cummins and Sartin (1987) noted that basal plasma insulin and insulin glucagon ratio increased in cows receiving a high fat diet containing 18 5% whole cottonseed. Also they found that response to bovine somatotropin (bST) at 50 d postpartum was decreased in animals receiving the high fat diet Glucagon, insulin, and insulin glucagon ratio in response to glucose infusion were not affected by diet, although plasma glucose peaked at greater concentrations in cows receiving the high fat diet, indicating possible insulin resistance

Glucose clearance from plasma after infusion of glucose into the jugular vein was lower and insulin release higher in cows fed high fat diets containing hydrolyzed animal-vegetable fat compared to control cows (Palmquist and Moser, 1981). The negative relationship between glucose utilization and insulin was linear, indicating insulin resistance in cows fed fat. Basal glucose and insulin levels were decreased by addition of fat to the diet

Hepatocytes of calves incubated with non-esterified fatty acids (NEFA) had increased hepatic triglyceride concentrations and gluconeogenic rates (Strang et al 1995a) and decreased insulin responsiveness and clearance rates (Strang et al 1995b),

indicating that FFA in plasma and triglyceride accumulation in the liver may induce hepatic insulin resistance

In another trial rapeseed oil was infused into the duodenum of early lactation and midlactation cows to examine the effects on plasma glucose, β-hydroxybutyrate, lipids and hormones (Gagliostro et al., 1991). Levels of glucose, β-hydroxybutyrate, and free glycerol in plasma were not affected by oil infusion. However, plasma NEFA, triglycerides, phospholipids, and both esterified and free cholesterol were increased by oil infusion compared to controls. Plasma NEFA were not affected by treatment prior to calving, but NEFA were higher in control animals by two weeks post calving. Few effects were noted for plasma hormones in early lactation cows, but in midlactation cows, plasma insulin was lower and bST and bST:insulin ratio were higher in animals receiving oil infusion.

Effects of Supplemental Fat on Milk Production and Composition

Effects of supplemental dietary fat on milk yield and composition have been variable. Some have reported increases in milk production (Anderson et al., 1979; DePeters et al., 1989, Moallem et al., 1997, Pires et al., 1997) but these increases have been modest in comparison with expected gains given the high energy density of fat (Clapperton and Steele, 1983; Jenkins and Jenny, 1989). Other researchers have reported no effect on milk yield (Hawkins et al., 1985, Lough et al., 1988; Mohammed et al., 1988; Martinez et al., 1991, Bertrand et al., 1998; Jenkins, 1998). One report noted a slight negative effect on milk yield (Hemar et al., 1986).

Changes in milk composition frequently have been reported. Response of fat content has varied from increasing (Kronfeld et al., 1980, Smith et al., 1981, Martinez et al., 1991), to decreasing (Lough et al., 1988, Mohammed et al., 1988, Schauff and Clark, 1989). Decreased protein concentration of milk with addition of fat to the diet is commonly observed, although this does not always result in decreased protein yield (Anderson et al., 1979, Horner et al., 1986, DePeters et al., 1989, Schauff and Clark, 1989, Wilks et al., 1991).

Possible reasons for these observations include effects of dietary fat on rumen fermentation and animal metabolism, discussed previously. Again, type of fat used and interactions with other diet components, principally fiber, play a major role in the variability of observed reponses. Staples et al. (1991) and Smith and Harris (1992) reviewed several studies and concluded that variation in response depended on the type of fat and forage used in the experimental diets. Fats fed in combination with corn silage result in decreased milk fat and increased milk protein concentrations, whereas the reverse occurs when alfalfa hay is the principal forage in a fat supplemented diet. Animal fats such as tallow tend to increase milk yield, while whole cottonseed, a common source of vegetable fat, does not appear to have a significant effect on milk production.

Vegetable sources

A number of fat sources have been tested for their effects on milk production and composition. Oil seeds such as whole soybeans and whole cottonseed have been used in a number of trials because they are sources of protein as well as fat.

Ruegsegger and Schultz (1985) studied the effects of heat-treated whole soybeans on performance of high producing, early lactation cows. Cows received either 0 or 25% heat-treated soybeans. Cows receiving soybeans reached peak production later than control cows (5 vs. 3 wk) but at higher levels of production (39.8 vs. 39.4 kg/d). By 15 wk, treated cows were producing 2.0 kg/d more than controls, suggesting improved persistency when soybeans are fed. Milk fat percentage increased on the whole soybean diet, while protein and casein levels in milk were unaffected by treatment.

Calcium soaps were added at either 3 or 6% of diet DM to diets containing extruded soybeans to examine the effects on milk production and composition (Schauff et al., 1992). Milk production was increased when extruded soybeans were added to the diet compared with controls (37.2 vs. 38.1 kg/d), but milk yield declined as CaLCFA were added to fat supplemented diets (3%, 37.0; 6%, 33.3 kg/d). No consistent effect of increasing fat on milk fat and protein could be detected in this study.

Milk yield responded quadratically to addition of 2 or 4% CaLCFA of canola oil fatty acids in a study by Chouinard et al. (1997). Milk yield was increased when 2% CaLCFA were added to the diet, but decreased when the level of CaLCFA increased to 4%.

The long term effects of feeding oilseeds were evaluated in another study based on data from previously reported trials (Schingoethe and Casper, 1991). One hundred ten cows which received either control (no added fat) or fat from sunflower or soybeans during wk 4 through 16 of lactation were evaluated for 305 d lactation performance. The authors reported that milk yield from wk 4 to 16 increased 2.8% when additional fat was

fed, while total lactation yield increased 3 6% for this group Percentages of both milk fat and protein were lower in cows which received fat, and continued to be less than that of cows which received control diets. This study confirmed earlier findings of Ruegsegger and Schultz (1985) that persistency was enhanced by feeding oilseeds in early lactation.

Canola seed was fed at different concentrations to lactating dairy cows to study the effects on milk fat and protein (Khorasani et al 1991) Canola seed which had been subjected to forced hot air to achieve an internal temperature of 145°C was fed to early lactation cows at levels of 0 2.5 5 0 7.3, and 9 6% of concentrate DM Effect on milk yield was non-significant, but tended to peak at 5% added fat. Milk protein percentage decreased linearly with increasing levels of canola seed Concentrations of medium- and odd-chain fatty acids in milk also declined linearly with increasing canola seed supplementation although overall fat content of the milk was not altered Palmitic acid (16 0) in milk decreased linearly for all but the highest level of canola supplementation, which was comparable to the control diet.

Changes in milk fatty acid profiles have been attributed to negative effects of fat on cellulolytic bacteria, as much of the medium-chain fatty acids are synthesized de novo in the mammary gland from acetate derived from ruminal metabolism Likewise, odd-chain fatty acids are produced by ruminal bacteria and incorporated directly into milk fat However, a study comparing canola oil and high oleic sunflower oil infused into the abomasum likewise resulted in a decrease in short , medium and odd-chain fatty acids in milk for both fat sources suggesting that effects of long-chain fatty acids on milk fatty acid profiles is post ruminal (LaCount et al., 1994) Uptake of preformed fatty acids from

the blood may be energy sparing compared to de novo synthesis in mammary cells Content of 16 0 also declined with increasing amount of oil infusion In this study, both milk yield and total fat percentage increased linearly with infusion of sunflower oil, while effect of level of canola oil on milk yield was not consistent. Total nitrogen (N) content of milk was decreased only at the highest level of infusion (400 g/d), with decreases in both casein and whey fractions, and increases in the non protein nitrogen (NPN) fraction

Canola seed has also been compared to flaxseed and Megalac® for their effects on milk yield and composition (Khorasani and Kennelly, 1994) Cows received either a control diet with no added fat, or 4% added fat from flaxseed, canola seed, or Megalac® Milk yields were 35 45, 32.94 32 18, and 36 19 kg/d for control, Megalac®, flaxseed, and canola diets respectively, but differences were not significant. Again, proportions of medium-chain fatty acids decreased and long-chain fatty acids increased in milk of cows fed the oilseeds but overall milk fat content was unaltered, confirming results of Khorasani et al. (1991) and Lu Count et al. (1994)

Whole cottonseed is a common dietary supplement to dairy cattle rations, particularly in the South, as they are abundant, inexpensive, and are a source of protein and effective fiber as well as fat Several studies have been conducted comparing the effects of WCS vs diets without supplemental fat or other fat sources.

Milk fat and protein were increased when cows were fed whole raw cottonseed compared to cows that received no supplemental fat (Pires et al 1997) as was milk yield, although milk yield increase was modest (17 6 vs 18 7 kg/d) Milk fat percentage declined when WCS were roasted compared to control (3 76 vs. 3 55%) although protein

percentage increased in this trial (3 19 vs. 3 28) However, these differences were not significant.

Cows supplemented with WCS produced more milk than cows supplemented with whole soybeans and soybean hulls in a study by Abel Caines et al (1997) Milk fat and protein were unaffected by fat source in this study which fed a combination of alfalfa and corn silages in a 45 55 ratio

Wu et al. (1994) compared the effects of whole cottonseed alone or mixed with either safflower oil or two levels of prilled tallow (2.2 or 4 4 %) Whole cottonseed alone had no effect on milk yield, while addition of safflower or prilled tallow increased milk yield over controls (35 0. 34 3 vs 32.5 kg/d) Milk fat percentage tended to be depressed by addition of safflower oil (3 26 vs 3 49), but increased when prilled tallow replaced safflower oil (2.2.3.58% 4 4 3.51%) Milk protein percentage was depressed for all fat supplements Yields of fat and protein, however were not greatly affected by treatment.

Animal fats

In general, trials designed to study the affects of animal fats have utilized tallow grease, and animal-vegetable fat blends. Recently, interest has been generated in fish oil and fish meal as fat supplements for dairy cattle. Reports of effects of animal fat on milk protein have been similar to those of vegetable oils

DePeters et al (1989) studied the effects of grease on milk nitrogen content in rations of varying energy density Grease was added at either 0 or 3 5% of ration DM in diets with either 1 6 or 1 7 Mcals net energy for lactation (NEL)/kg Adding fat to the diet resulted in depressed total milk N, casein N_2 and whey protein concentrations at both

energy densities compared to no fat diets. The high concentrate diet (1.7 Mcal) resulted in the highest concentration of milk N. Added fat increased the proportion of long-chain fatty acids, principally 18:0 and 18:1ω9, in milk fat and decreased short- and medium-chain fatty acids. Milk yield was higher for the high energy fat diet (3.5 % 1.7 Mcals) than for the other three diets (26.8 vs. 23.4 and 24.9 kg/d), which were similar. Total milk fat was higher for the lower energy fat diet, which the authors attributed to the higher proportion of forage in the diet.

Fish meal has also received attention in recent years, primarily as a source of ruminal bypass protein, but also more recently as a source of very long-chain PUFA, which appear to be relatively resistant to ruminal degradation, as discussed above. However, addition of fish meal to the diets of dairy cows depressed milk fat percentage and yield in a linear fashion, even though ruminal fermentation patterns were not affected (Spain et al., 1995). Dry matter intake showed a quadratic response to increasing levels of fish meal in the diet, but differences in intake were not sufficient to alter milk production, the authors concluded. Milk protein concentration was not affected by fish meal in this experiment.

Fish meal was compared to fish oil infusion (Spano et al., 1995). Although fish meal increased the levels of n-3 fatty acids in plasma compared to fish oil, no changes were found in milk yield or composition due to treatments. Cows fed fish meal or fish oil did not differ in ruminal VFA patterns or milk fat yield.

Hydrogenated tallow was added to diets at levels of 0, 2, 4, or 6 percent to examine effects on milk yield and composition in an experiment by Brackley and Elliot

(1993) Milk yield and DMI were not significantly different among treatments. Tallow decreased milk protein compared to controls (2.84 vs. 3 02% CP), but the effect was not linear Milk fat tended to decrease linearly with increasing tallow in the diet However, milk fat percentages were low for all diets in this trial (3 09 2 97 2.94, and 2 90% for control, 2 4, and 6% fat, respectively) The authors speculated that decreased milk fat yield might be due to inadequate fiber particle size (ensiled forages were used) or high levels of *trans*18 1 in the partially hydrogenated tallow *Trans* fatty acids infused abomasally depressed milk fat percentage compared to *cis* fatty acids and control in a study by Romo et al (1994)

Elliott et al. (1993) fed diets containing either no fat, high oil corn (HOC) replacing regular corn grain, high oil corn plus 2.5% tallow, and high oil corn plus 5% tallow Milk yield tended to be greater for cows on high oil corn plus 2 5% tallow, but the differences were not significant Yields of milk fat and protein were unaffected by diet, although protein percentage was lower for cows receiving tallow (3 04 2 98 2 87 2 80% for control, HOC, HOC plus 2.5% tallow and HOC plus 5% tallow, respectively) Milk fat percentage increased when HOC replaced regular corn, but decreased when tallow was added to the diet, this result was significant for HOC vs tallow but not for other treatments.

Milk yield was greatest for cows receiving 1 6 % of DM as tallow compared to cows consuming 8% of DM as roasted soybeans (Grummer et al., 1996) However, cows on tallow diets also produced more protein and fat corrected milk (FCM) than cows receiving hydrolyzed tallow fatty acids or hydrogenated tallow

Trans fatty acids

Recent evidence indicates that *trans* fatty acids resulting from the biohydrogenation of unsaturated fat in the rumen may have negative effects on milk fat production. In lactating rats fed 25% of dietary fat as *trans* isomers of 18:1, *trans* isomers depressed milk fat (Teter et al., 1995). In dairy cattle, milk fat depression occurred within three days of beginning feeding of 5% of DM as high 18:2ω6 soybean oil, and this depression persisted throughout the treatment period. At the same time, *trans* fatty acids in milk increased in cows fed with oil (Piperova et al., 1995).

Erdman and Teter (1995) noted that *trans* fatty acids in milk were negatively correlated with milk fat percentage in dairy cattle, and that post-ruminal infusion of *trans* 18:1 fatty acids resulted in similar decreases in milk fat. Adding buffers to the diet decreased *trans* fatty acid concentration and increased total milk fat percentage in cows fed high concentrate diets, while cows receiving the same diets without buffer produced more *trans* fatty acids and less total milk fat (Kalscheur et al., 1995).

Wonsil et al. (1994) also reported an inverse relationship between milk fat concentration and *trans* fatty acid flow to the duodenum in dairy cattle. Cows were fed diets containing either no added fat or 3% fat as hydrogenated tallow, menhaden fish oil plus 18:0 (1:1), or soybean oil plus partially hydrogenated soybean oil (1:1). Milk fat yield was lower (0.90 and 1.14 kg/d) and amount of *trans* fatty acids higher (163 and 152 g/d) in the fish and soybean oil diets compared to control and hydrogenated tallow (milk fat, 1.22 and 1.17; trans18:1, 37 and 38 g/d), and milk fat decreased linearly with amount of *trans* fatty acids in the duodenum and milk for all diets.

Infusion of fat high in *trans* fatty acids (93% hydrogenated vegetable shortening plus 7% corn oil) into the abomasum of dairy cows also resulted in decreased milk fat compared to cows infused with mainly *cis* fatty acids (65% high oleic sunflower oil and 35% cocoa butter) (Gaynor et al., 1996). This occurred despite the fact that other blood metabolites and hormones (NEFA, glucose, triglycerides, and insulin) were not different between treatments.

The relationship between *trans* fatty acids and milk fat depression is not perfectly correlated, however. In a study by Kalscheur et al. (1997a), fat from high oleic sunflower oil, high linoleic sunflower oil, and partially hydrogenated vegetable shortening all depressed milk fat, although *trans* fatty acid composition of duodenal digesta and milk differed among treatments.

Adding buffers ($NaHCO_3$ and MgO) to diets has been shown to at least partially prevent milk fat depression in high corn diets (Kalscheur et al., 1997b). High grain diets generally reduce rumen pH and may hinder complete hydrogenation of unsaturated fatty acids, resulting in increased *trans* fatty acid production and escape from the rumen. This experiment, however was carried out in diets without supplemental fat. Supplementation of diets containing 4% CaLCFA of canola oil plus buffers tended to alleviate milk fat depression even in the presence of increased *trans* fatty acids (Chouinard et al., 1997).

The mechanism by which *trans* fatty acids result in reduction of milk fat percentage have not been elucidated.

Interactions with other dietary components

As noted earlier much of the variation in response to fat feeding, particularly in the fat and protein content of milk, has been attributed to interactions between fat and other components in the diet. Interactions between dietary fat and protein and forages have been considered in a number of trials

Fiber When Megalac® and roasted soybeans were fed in combination with soyhulls to replace concentrate, milk production and feed efficiency improved, but milk protein percentage decreased (3 16 vs. 2.86% for control vs SH plus fat) (Firkins and Eastridge 1992) Protein yield was depressed with addition of fat and SH to the diet (1 150 vs. 1 127 kg/d for control vs SH plus fat), but this difference was not significant. The authors concluded that a 13 to 14% increase in efficiency of milk production resulted from addition of fat to the diet. Since only one added fat diet was fed, containing both soybeans and Megalac® it cannot be determined whether the oilseeds or ruminant fat, or both contributed to the depression in milk protein concentration in this study

One possible explanation for interactions between fiber and fat relates to the particle size of the forage. It has been theorized that smaller particles, typical of silages, become coated with fat, preventing bacterial attachment and subsequent breakdown of the roughage, while larger particles are less coated and offer more sites for enzymatic binding

To test this theory Grant and Weidner (1993) added whole roasted soybeans (WRS) to diets containing either finely chopped alfalfa silage with or without coarsely chopped alfalfa hay In diets containing silage plus hay, WRS decreased milk yield compared to diets without WRS (25 5 vs 30.1 kg/d), but increased fat concentration

(4 07 vs 3 80%). Milk protein was also depressed when WRS was added to the diet containing hay compared to other diets (2 95 vs. 3 06 3 07 and 3 19% for silage-WRS silage+WRS, silage+hay-WRS, respectively)

In a second experiment, the authors compared the effects of different levels of NDF in alfalfa and corn silage diets when WRS was fed (Grant and Weidner 1993) In this study, fat did not affect milk yield or composition, but low NDF (25%) decreased fat concentration compared to the high NDF (29%) diet (3 57 vs 4 01 and 3 91%) An interaction ($p<.10$) was noted between fat and NDF content of the diet. Addition of WRS to diets with 25% NDF decreased milk yield compared to the same fiber level without added WRS (30 9 vs. 33 1 kg/d) The opposite results were obtained with 29% NDF diets with or without added WRS (32 1 vs 30 7 kg/d)

No interaction between ruminally protected fat as CaLCFA and diet NDF level for milk production or composition were noted in a trial by Canale et al (1990a) Neutral detergent fiber levels were 25 or 31% of diet DM (50 or 70% alfalfa silage) Calcium soaps depressed milk protein percentage regardless of NDF content of the diet (25% NDF-CaLCFA, 3 05%, 25% NDF+CaLCFa, 2 98%, 31% NDF-CaLCFA, 3 15%, 31% NDF+CaLCFA, 3 07%) Milk fat percentage was increased by CaLCFA for higher levels of NDF (31% NDF-CaLCFA, 3 54%, 31% NDF+CaLCFA, 3 66%)

The interaction of forage type and fat source were studied in two trials (Smith et al., 1993, Adams et al , 1995) to better understand variations in response to fat feeding In the trial conducted by Smith et al (1993), corn silage was fed either as the sole source of roughage in the diet or replaced with 25 or 50% of forage as alfalfa hay Fat sources

were control (no fat), 2 5% tallow, 12% WCS, or 2.5% tallow plus 12% whole cottonseed. Milk (21 0 vs. 22.8 kg/d) and fat yields (666 vs. 809 g/d) were depressed when WCS was fed with corn silage alone compared to whole cottonseed diets when alfalfa hay was added

In contrast with previous studies (Clapperton and Steele, 1983, Knapp and Grummer, 1990) diets with added tallow depressed milk yield regardless of forage mixture. Fat was depressed more when corn silage was the only forage compared to diets with added alfalfa.

In a subsequent study alfalfa hay bermudagrass hay and cottonseed hulls were used to replace a portion of corn silage in diets containing either an added fat, 12 5% whole cottonseed, or 2 5% tallow (Adams et al 1995) In this trial, addition of alfalfa hay did not alleviate milk yield or fat depression in diets with WCS Milk yields were highest for diets containing cottonseed hulls (27 03 kg/d) regardless of fat treatment Whole cottonseed depressed milk yield (25 36 kg/d) compared to tallow and control diets which were similar (26 37 and 26 48 kg/d) A significant interaction was noted between fat source and forage type for milk protein. On diets with corn silage as the sole forage, whole cottonseed depressed protein percentage compared to tallow and control diets (2.90 vs 3 07 and 3 06%) However, when bermudagrass hay replaced a portion of the corn silage, tallow depressed protein percentage compared to whole cottonseed and control (2 98 vs 3 19 and 3 18%) (P=0 038 for interaction)

Because forage:concentrate ratio of the diet was lower in this trial compared to the trial by Smith et al (1993), alfalfa hay comprised only 11.25% of the diet The minimum

level of substitution in the trial of Smith et al (1993) was 12.5% of DM as alfalfa hay The amount of alfalfa or total dietary fiber may have been inadequate to alleviate the negative effects of WCS in the study by Adams et al (1995). The strong positive effects of cottonseed hulls on milk production and composition do not support the theory that forage particle size is a determining factor in the interaction between fat and fiber

The order in which fat is added to the diet may also affect the interactions that apparently occur between fat and fiber In a study by Drackley et al (1994) tallow added directly to forage tended to increase milk yield compared to diets where fat was added first to concentrate or last to TMR (39 0 vs. 38 6, 37 8 kg/d) Milk fat, on the other hand, tended to be depressed when fat was added to haylage or last to TMR compared to mixing first with concentrate (1 15 vs. 1.21 kg/d). Milk protein was not affected by method of mixing, and tended to be higher for diets containing tallow compared to control diets (1 16 vs. 1 07 kg/d) in contrast to several studies (Drackley and Elliott, 1993, Elliott et al., 1993 Adams et al., 1995)

Protein. Amount and rumen availability of dietary protein may also influence milk production and composition responses to fat Andrew et al (1991) fed two levels of crude protein (16 and 20%) with or without CaLCFA to lactating dairy cows to examine the effects on milk production Milk yield increased at both levels of protein when CaLCFA were added to the diet. Increasing the protein concentration of the diet did not alleviate the depression in protein percentage when CaLCFA were fed (16% CP-CaLCFA, 3 23%, 16% CP+CaLCFA, 2.97%; 20% CP-CaLCFA, 3 13 %, 20% CP+CaLCFA, 2.96%). Calcium soaps had no significant effect on milk fat at either level of CP

Klusmeyer and Clark (1991) fed diets with either soybean meal or 50:50 soybean meal:fish meal as protein sources, with or without added Ca-LCFA. A significant interaction occurred between fat and protein source in weight percentage of 6 and 8 carbon fatty acids in milk. Addition of fat to diets containing fish meal depressed content of these fatty acids compared to diets containing Ca-LCFA and soybean meal.

Significant interactions occurred when dietary fat and ruminally protected amino acids were fed to lactating cows in another study (Cunale et al., 1990b). Milk fat yield was depressed by addition of either animal vegetable fat or ruminally protected methionine and lysine, but increased when the two were fed together (1.21, 1.13 vs. 1.30 kg/d). Milk protein percentage was increased by addition of protected amino acids compared to control (3.11 vs. 3.02%), but depressed by added fat (2.94%). Protein percentage was not different between controls and fat plus amino acids (3.02 vs. 3.05%). Milk yield was increased by adding fat compared with control (34.7 vs. 33.5 kg/d), but amino acids had no effect on milk production either alone or in combination with fat.

Post ruminal infusion of crude protein in the form of blood meal or corn gluten meal did not affect milk fat or protein percentages in fat fed cows, but did increase fat and protein yields due to increased milk yield (Goodling and Grummer, 1990).

In contrast, ruminally protected methionine and lysine increased total milk N and casein N yields in diets containing 3.9% yellow grease, but had no effect on total N or protein fractions in diets without added fat (Chow et al., 1990). Addition of protected amino acids did not affect milk fat percentage or fatty acid composition, but fat content of milk was higher in diets containing fat compared to isocaloric diets without fat.

Jersey cows fed WCS with or without rumen protected methionine and lysine did not differ in DMI or milk yield from cows receiving a no supplemental fat diet (Bertrand et al 1998) Milk fat tended to decrease for cows receiving WCS, and milk protein was also depressed by feeding WCS alone. Milk protein was increased in cows receiving WCS plus rumen inert amino acids, suggesting possible interference by WCS on provision of lysine and/or methionine to the small intestine.

Feeding a combination of rumen undegradeable intake protein (UIP) coated with Ca LCFA enhanced milk yield, feed efficiency and increased milk fat content in an experiment by Sklan and Tinsky (1993) Milk yield during the feeding trial was increased by feeding the fat-protein product compared to controls (42.5 vs. 41 5 kg/d), as was total lactational yield (10,679 vs 10,188 kg) Milk fat yield also increased in response to feeding the product compared to controls both during the trial (1 39 vs 1 31 kg/d) and for the total lactation (335 8 vs. 315 1 kg). Daily milk protein yield was unaffected during the trial but total lactation protein yield was greater for cows that had received the product (307 4 vs 295 8 kg)

Milk production was also higher for cows receiving combinations of tallow, molasses (source of readily fermentable carbohydrate) and bypass protein (blood meal, meat and bone meal, and corn gluten meal 5% of DM) compared to animals receiving bypass protein alone (Mutsvangwa and Schingoethe, 1997) However, milk protein percentage was higher for cows receiving molasses plus bypass protein compared to those receiving fat plus bypass protein

To better understand the mechanism(s) controlling fat effects on milk production, Palmquist and Moser (1981) conducted two experiments measuring the effects of fat on plasma insulin, glucose utilization and milk protein content. In the second of these experiments a calcium soap of soybean oil was fed to Jersey cows in a 4x4 Latin square design. Diets did not influence milk yield, but concentration and quantity of milk fat increased when the fat supplement was fed. Lipid concentration of plasma increased, while insulin and glucose concentrations were depressed. Milk protein percentage decreased when fat was fed compared to the no fat diet (3.86 vs. 3.95%), but protein yield was unaffected due to the increase in milk produced. Palmquist and Moser (1981) questioned the theory that the decreased microbial synthesis could result in decreased milk protein, and suggested that nutrient availability and synthetic processes at the level of the mammary gland might be responsible.

Infusing fatty acids into the abomasum of lactating cows decreased milk yield and both percentage and yield of CP and N fractions in milk compared with animals infused with meat solubles (Christensen et al., 1994). However, cows infused with mostly saturated fatty acids produced more milk (32.8 vs. 30.6, 30.2, and 29.4 kg/d), fat (1.09 vs. 1.08, 1.00, and 0.94), protein (1.01 vs. 0.96, 0.92 and 0.88 kg/d) and casein (0.70 vs. 0.67, 0.64 and 0.61) than cows infused with mostly unsaturated fatty acids from canola, soybean or sunflower oils.

These findings support the theory of Palmquist and Moser that effect of fat on milk composition may occur at the level of the mammary gland. Milk fatty acid profile

reflected those of the fatty acids infused, supporting the theory that fatty acids are taken up from the blood by mammary cells in preference to de novo synthesis.

Infusion of rapeseed oil into the duodenum of mid-lactation cows increased mammary apparent uptake of triglycerides and esterified cholesterol compared to control cows (Gagliostro et al., 1991). However, in early lactation cows, mammary uptake of these metabolites was not different between control and oil infused cows. McNamara (1989) noted that in early lactation lipogenesis and lipolysis proceed simultaneously at high rates such that fat supplementation may have little effect on fatty acid profiles in milk.

Also in the mid-lactation trial, insulin levels in plasma were decreased and somatotropin increased in oil infused cows compared to controls. In the early lactation trial, however, plasma insulin and somatotropin were unaffected by treatment. Insulin is needed for uptake of glucose by peripheral tissues, but not by the mammary gland. Also insulin stimulates lipogenesis and suppresses lipolysis in tissues. Conversely, somatotropin depresses lipogenesis and increases partitioning of lipids to the mammary gland.

Unfortunately preprandial and postprandial N and amino acids were not measured in this trial. It has been suggested that fat may interfere with mammary uptake of amino acids and peptides, thus reducing protein synthesis (Cant et al., 1993).

Nutrition and Management to Enhance Reproduction

Evidence has accumulated in recent years to indicate that supplemental dietary fat may also enhance conception rates in early postpartum cows. Possible mechanisms for these effects include reducing the severity of negative ES, increasing the amount and availability of the hormonal precursor cholesterol, and modifying prostaglandin synthesis.

As discussed previously, negative ES is a common state in early postpartum cows when the energy requirements of milk production exceed energy intake. To survive, animals in nutritional deficit prioritize the partitioning of nutrients, often decreasing or temporarily ceasing certain activities or functions. This state has been termed homeorhesis (Bauman and Currie, 1980). Theoretically, survival would have greatest priority, followed by maintenance of the current offspring (milk production), while future reproductive activity would have lower priority.

The period to first ovulation may be critical for timing of conception. The likelihood of conception to insemination increases with increasing number of ovulatory cycles prior to breeding (Butler and Smith, 1989). If days to first ovulation are prolonged, fewer ovulatory cycles will occur prior to first insemination, thus decreasing the probability of conception. Thus, then, may explain the relationship between ES and reproductive capability.

Negative ES has been found to have a strong positive correlation with days to first ovulation which apparently becomes established within the first two weeks of lactation (Butler and Smith, 1989). The relationship between NES and days to first ovulation is complex, however. In studies by Butler and Smith (1989), first ovulation occurred

approximately 10 d after maximum NES had been reached, before positive energy balance (PES) was achieved but as the animal was recovering from NES Based on these findings, Butler and Smith (1989) suggest that both magnitude and recovery rate are important in the relationship between ES and first ovulation

Villa-Godoy et al. (1988) studied the relationship between NES and ovarian activity during the first two weeks postpartum in both primiparous and multiparous Holstein cows Duration of luteal phase was not associated with ES However ES within 9 d of calving was positively correlated with plasma progesterone (P_4) concentration during second and third postpartum luteal phases In support of the observations of Butler and Smith (1989), interval to nadir and magnitude of NES interacted to decrease plasma P_4 concentrations during the second and third postpartum estrus cycles in this study Energy status was related more to intake, and only to a lesser extent correlated with milk yield Neither was milk yield correlated with mean or peak P_4 levels or duration of luteal phases. In contrast to Butler and Smith (1989) Villa-Godoy et al (1988) suggested that the factors which mediate effects of NES on luteal function occur after or persist beyond nadir of NES

Energy status appears to be less critical for heifers than it is for cows. In another experiment by Villa-Godoy et al (1990), the effects of ES and body condition on estrous cycles and estrus behavior in heifers were studied. Neither NES nor over conditioning reduced peak or duration of estrous behavior However, onset of diestrous was delayed in fat heifers in NES compared to other animals The authors suggest that NES in over conditioned animals may result in reduced accuracy of timing artificial insemination The

authors point out, however, that magnitude of NES is 5 to 10 times greater in lactating cows than in induced NES heifers. Domecq et al (1997) performed a multiple logistic analysis based on records of 720 Holstein cows which revealed that the top three ranking variables as predictors of conception rate to artificial insemination (AI) were lactation number, milk yield at 120 days in milk (DIM) and body condition score (BCS). Decreased BCS in the first month of lactation was associated with decreased likelihood of conception, while increased milk yield at 120 d was associated with an increased likelihood of conception.

Rasby et al. (1991) studied the impact of nutrient intake and body condition on reproductive function in Hereford cows. Concentration of luteinizing hormone (LH) in the pituitary was not affected by nutrient intake or body condition. However, thin cows had higher LH concentrations in serum after injection of gonadotropin releasing hormone/thyroid releasing hormone (GnRH/TRH) than did cows with moderate or fat condition scores. Wet ovarian and corpora lutea weights were greater for fat cows compared to other groups. Percentage of body fat in thin cows that did not exhibit luteal activity was 4.1% compared to 7.4 % for thin cows with luteal activity. Thin cows had the least total follicular fluid, while fat cows had the most.

Whether the above factors are related to actual reproductive responses may be debatable. In a study by Rugg et al. (1992), BCS at calving did not affect number of days to first observed estrus or first service. In fact, cows calving with condition scores greater than 3.5 required more days to conceive compared to cows with condition scores

less than 3 5 However cows losing more than 0 75 points of condition also had delayed conceptions, but this difference was not significant.

Milk yield may also impact reproductive function at least indirectly through its effects on body condition loss in early postpartum cows, according to the study by Ruegg et al (1992) Cows producing above the mean for 305 d lactation required more services per conception compared to cows producing below the mean. Incidence of ovarian follicular cysts were also increased for higher producing cows, although overall incidence of follicular cysts were greater in this study than considered normal (39 vs 10%) Energy status did not appear to be related to diagnosis of follicular cyst in this study Rather an interaction occurred between serum cholesterol concentration and incidence of follicular cysts between 30 and 45 d postpartum. Cows with cysts had greater plasma cholesterol concentrations than cows that did not develop cysts the authors concluded This may be due to greater mobilization of body stores during this time period to support milk production. Energy status was not calculated in this study

The relationship between high milk yield and infertility is not clear It has been proposed that genetic selection for high yielding animals is negatively correlated with reproduction. However, studies have failed to show a genetic relationship between productive and reproductive capacity Indeed, it has been shown that heritability of fertility is nearly zero (Hansen et al., 1983) Alternatively, the metabolic stress of producing large quantities of milk may compromise other body functions.

Some studies have found a positive relationship between milk yield and days to first ovulation but this has not been consistent In their review Butler and Smith (1989)

stated that the relationship between milk yield and days to first ovulation only becomes significant after d 40 of lactation, when most cows have already ovulated.

The negative correlation between milk yield and ovarian activity is not always observed. In a study by Staples et al. (1990) 54 early lactation cows were categorized according to their DMI, milk yield, energy status, and reproductive function during a 9 wk study. Fifteen of these cows were anestrus during the full term of the study, and were compared with two cycling groups: one group of 25 cows with corpora lutea forming within 40 d after parturition and 14 with corpora lutea between 40 and 60 d postpartum. In contrast to Ruegg et al. (1992), Staples et al. (1990) observed that anestrus cows ate less feed and produced less milk than either of the two cycling groups. However, anestrus cows did lose more body weight than cycling cows, resulting in greater NES. Cows cycling before d 40 postpartum were in less NES than either anestrus cows or cows cycling after d40.

Contrary to Lucy et al. (1991a), no relationship between ES and estrus activity was noted before d 40 postpartum. Carryover effects of NES were noted for anestrous cows in that, although all groups had achieved ES by the fifth week after calving, cows in this group did not begin cycling until after 9 wk postpartum. Also, conception rate among cows in this group was only 33.3%, compared to greater than 80% for the other groups.

Days to first visual estrus and number of ovulations before first observed estrus were greater in higher producing cows (10,814 kg) than average cows (6,912 kg) in a study by Harrison et al. (1990). First observed estrus for the high group occurred at 66 d vs. 43 d for the average group, and number of ovulations prior to first observed estrus was

1 6 for the high group compared to 0 7 for the average group High producing cows experienced more NES in the first two weeks of lactation than the average group which supports the suggestion of Staples et al. (1990) that NES early postpartum may have carryover effects Harrison et al. (1990) concluded that although both groups resumed cycling at about the same time postpartum, expression of estrus differed between groups

Dietary Fat Effects on Reproduction

In a study designed to elaborate the relationship between ES and early postpartum reproductive function, Lucy et al (1991b) fed cows diets differing in type of forage with or without added CaLCFA. As ES increased, the number of small ovarian follicles (6 to 9 mm) decreased, while larger follicles (10 to 15 mm) increased. They concluded that before d 25 postpartum, ES explained the differences observed between treatments, while the relationship did not appear to hold after d 25

Cows receiving CaLCFA at a rate of 2 2% of the diet DM achieved PES earlier than cows consuming diets without CaLCFA in an experiment by Garcia-Bojalil (1993) Cows in this study were fed two levels of degradable intake protein (DIP)(55 and 76%) with or without CaLCFA. Cows receiving diets containing the fat supplement and less degradeable protein recovered body condition more rapidly than animals on other diets By contrast, cows consuming the highly degradable protein diet without fat remained in NEB longer than other treatment groups, most likely due to depressed DMI in this group

Uterine involution was more rapid in cows receiving the less degradeable protein diet, which contained fish meal as one of the UIP sources. The author suggested that the more rapid rate of involution may be due either to the diminished loss of protein reserves

during early lactation from tissues including the reproductive tract, or to the provision of long-chain PUFA from fish meal as precursors of $PGF_{2\alpha}$.

Additionally, cows consuming diets with CaLCFA had higher second service conception rates than cows without the supplement (55%DIP, 66.7 vs. 37.5%; 76%DIP, 83.3 vs. 20.0%) while first service conception rates also were numerically, though not significantly, higher for the fat supplemented cows (55%DIP, 45.4 vs. 27.3%; 76%DIP, 45.5 vs. 40.0%). Ferguson et al. (1990) also noted improved conception rates among dairy cows receiving grilled fat as 2% of the diet DM.

Sklan and Tinsky (1993) also studied the effects of feeding CaLCFA on ovarian function in early postpartum cows. In contrast to Garcia-Bojalil (1993), they noted that BW decreased more and reached nadir later in fat fed cows compared to control animals (d 32 vs. d 12). Also, ovarian cyclicity commenced later in cows receiving the CaLCFA supplement than unsupplemented cows. Forty-two percent of control cows were cycling by d 20 compared to 21% of cows receiving CaLCFA. However, once cycling commenced, a greater proportion of cows in the fat-fed group had normal length cycles than cows that did not receive fat. Also, as in Garcia-Bojalil (1993), conception to second and later services was greater among cows receiving fat compared to controls (42.6 vs. 25.0%), and more cows in the fat fed group were pregnant by 150 d (82.4 vs. 62.5%), with fewer days open than control cows (115 vs. 149 d).

A follow up study by this same group (Sklan et al., 1994) noted that conception to first AI was lower among primiparous cows receiving CaLCFA compared to controls, but no differences were noted for later services among any of the groups. Again, body weight

loss was greater among cows receiving CaLCFA than control animals, and this trend was more apparent and longer lasting in primiparous animals compared to multiparous cows Dry matter intake did not differ between groups in either study, but cows fed CaLCFA produced more milk than controls in both studies, indicating that preferential nutrient partitioning to the mammary gland may be responsible for the prolonged period of NES

Prilled fatty acids were fed to early postpartum dairy cows to examine the effects of fat on ES, plasma cholesterol, Insulin-like growth factor 1 (IGF-1), and ovarian follicular dynamics in early postpartum cows (Beam and Butler, 1998) No differences were noted for any of these measurements between fat-fed cows and no-fat controls Regardless of treatment, daily ES was positively correlated with serum IGF-1 and negatively correlated with plasma NEFA Cows with non-ovulatory first wave follicles from d 8 to 14 postpartum had a longer interval to ES nadir, lower IGF-1, and higher milk yield than cows which ovulated within the first two weeks postpartum

Fat Supplementation and Reproductive Endocrinology

Dietary fat has been shown to stimulate endogenous cholesterol synthesis in many mammalian species. Fat absorbed from the intestines is transported to the liver where it is packaged, along with cholesterol mostly in the form of cholesteryl esters of fatty acids in a carefully controlled ratio, into very low density lipoproteins (VLDL) and released into the blood stream These lipoproteins are reduced to low density lipoproteins (LDL), primarily by the liver, by removing some of the triglycerides Low density lipoprotein particles are small enough to pass into the interstitial space where they bind with receptors on peripheral cells and are taken up by endocytosis Triglycerides and cholesterol are then

either stored or utilized by the cell for energy or synthetic processes (Voet and Voet, 1995)

Although corpora lutea cells have the capacity to synthesize cholesterol *de novo* by far the majority of cholesterol utilized for steroidogenesis is provided via LDL and high density lipoprotein (HDL) particles (Niswender and Nett, 1994) Theoretically, increasing dietary fat to cattle should also increase plasma cholesterol and enhance production of progesterone through providing more of its precursor

Increases in plasma cholesterol have been observed, but this has not always resulted in increases in progesterone (Carroll et al , 1990; Carroll et al , 1992, Ryan et al – 1992)

Addition of fat as prilled long-chain fatty acids (0 or 5% of diet DM) to diets with differing forage:concentrate ratios (45 55 64 36, or 84 16) increased plasma cholesterol levels in multiparous, early lactation cows (Carroll et al , 1990) Plasma P_4 was higher in fat supplemented cows during mid- and late luteal phases of the second postpartum cycle, and during metestrous, to early luteal phase and mid to late luteal phase of the third cycle However, no treatment differences were noted in P_4 synthesis during the follicular phase and P_4 production was actually decreased in metestrous to early luteal phase of the first cycle in fat supplemented cows. Cows receiving supplemental fat also had higher plasma P_4 levels after AI, but no differences were detected between treatments for days to first observed estrus first service conception rates, or services per conception

Carroll et al. (1992) incubated isolated corpora lutea with various lipoprotein fractions in two experiments. In the first experiment, blood was collected from six heifers

and HDL were separated according to their ratio of cholesterol to protein (C:P). Addition of 20 and 200 μg of high C:P HDL increased P_4 production over low C:P HDL. At the 20 μg level P_4 levels increased from 625 ng/μg DNA for low C:P ratio to 900 ng/μg for high C:P. When the amount of HDL added to cultures was increased to 200 μg, P_4 levels were 1200 ng/μg for low C:P and 1500 ng/μg of DNA for high C:P ratios.

In the second of these experiments (Carroll et al. 1992) LDL and HDL from blood of pregnant, primiparous cows fed either 0 or 7% saturated long-chain fatty acids were separated and added to corpora lutea cultures at levels of 1, 10, 20, 50, 100, or 200 μg/ml medium. Fat supplementation did not alter plasma concentrations of LDL, but HDL concentration increased from 65.8 mg for plasma of unsupplemented cows to 79.8 mg for cows receiving 7% supplemental fat. Progesterone production by luteal cells increased linearly with concentration of cholesterol from LDL or HDL. No difference was observed between particle densities in ability to stimulate progesterone synthesis (Carroll et al., 1992).

Oldick et al. (1997) compared abomasal infusions of glucose with fat as either tallow or yellow grease for their effects on blood constituents and follicle dynamics in cows in PES. Cows infused with fat had higher plasma cholesterol than those infused with glucose, but plasma P_4 levels were unaffected by treatment in this study. However polynomial regression curves on P_4 concentration over time indicated that P_4 peaked higher for fat sources than for glucose infusion (11.7 vs. 9.3 ng/ml at d 12.5). Type of fat did not influence plasma P_4. Energy infusions decreased estradiol-17β concentrations of plasma compared to control (water infusion). The difference in estradiol-17β

concentrations was attributed largely to the effects of fat infusion. Cows infused with yellow grease had lower prostaglandin F metabolite (PGFM) following oxytocin challenge than other animals. The author concluded that yellow grease completely inhibited post oxytocin injection increase in $PGF_{2\alpha}$.

Yellow grease contains approximately 17.5% 18:2ω6. Linolenic acid is a precursor of 20:4ω6, which may be converted to 2-series prostaglandins such as $PGF_{2\alpha}$. However, diets high in 18:2ω6 inhibit 20:4ω6 synthesis when fed to preruminant calves (Jenkins, 1988). Intravenous infusion of soybean oil emulsion containing approximately 50% 18:2ω6 increased plasma PGFM concentrations in Holstein heifers (Lucy et al.,1990).

The mechanism for inhibition of prostaglandin synthesis by high 18:2ω6 concentrations has not been elaborated fully. It has been suggested that increased concentrations of substrate for prostaglandin endoperoxide synthase may increase the rate of a suicide reaction in which an inactive enzyme intermediate is formed. Linoleic acid and n-3 fatty acids may compete with 20:4ω6 for binding with cyclooxygenase, one of the rate limiting enzymes in prostaglandin synthesis. Alternatively, peroxides might accumulate, resulting in inactive enzymes (Oldick, 1994).

Mean number of follicles was not affected by treatment in the study by Oldick (1994). However, maximum size of the corpus luteum (CL) tended to be 2 mm smaller, and CL disappearance was delayed in cows receiving fat infusion compared to glucose infusion. Reduction in P_4 concentrations to <1 ng/ml was also delayed by fat infusion. These effects were associated with yellow grease infusion rather than tallow. Also, CL tended to emerge later and P_4 rose later in cows infused with yellow grease infusion.

compared to tallow infused cows The delay in development of the CL in yellow grease-infused cows was attributed to prolonged dominance of the first wave dominant follicle in cows receiving yellow grease. During recruitment phase of the second wave dominant follicle, glucose infusion resulted in a greater number of 6 to 9 mm follicles compared to fat infusions, such that fat infusion appeared to decrease the pool from which the ovulatory follicle might be recruited This may also be a result of the delayed stream of the first wave dominant follicle in cows receiving fat

Diets containing 5 4% soybean oil were found to increase the number of follicles between 5 and 9 9 mm in size when fed to beef heifers treated with follicle stimulating hormone (FSH) (Ryan et al , 1992) Again, supplemental fat increased cholesterol and P_4 concentrations in follicular fluid Concentrations of estradiol-17β in follicular fluid were unaffected by treatment in this study However, granulosa cells recovered from ovaries of fat-treated cows produced less estradiol 17β and more P_4 *in-vitro* than cells of cows receiving the unsupplemented diet. Diet had no apparent effect on number or characteristics of recovered embryos

Gossypol

Studies involving the effects of WCS on reproduction are limited and focus primarily on the potential negative effects of gossypol. Gossypol is a polyphenolic yellow pigment that is produced naturally in cottonseed as a defense against insect pests (Berardi had Goldblatt, 1980) It has been documented to be toxic in nonruminant and preruminant calves, causing extensive liver damage and death (Holmberg et al 1988, Risco et al , 1992) Effects of gossypol on adult ruminants is less evident, although toxicities have

been noted in lactating dairy cows (Lindsey et al., 1980; Smalley and Bicknell, 1982) and sheep (Morgan et al., 1988).

Brahman bulls fed WCS grew more slowly and reached puberty at an older age than bulls fed soybean meal or cottonseed meal in a study conducted by Chase, et al. (1994). Daily free gossypol intake by bulls averaged 0.03 g/d for soybean meal diets, 1.8 g/d for cottonseed meal diets, and 50.8 g/d for WCS diets. By d 196 of the experiment, bulls fed diets containing gossypol tended to have lower BW than bulls receiving soybean meal, while bulls on the highest level of gossypol (WCS diet) had the lowest BW (325 vs 355 kg). Bulls fed soybean meal had higher percentage of motile sperm compared to cottonseed meal and WCS fed bulls (73 vs. 62 and 69%). However, percentage of motile sperm were higher on the WCS diet compared to the cottonseed meal diet.

Two stereo isomers of gossypol (+ -) have been identified by high performance liquid chromatography and are believed to differ in their biological activity. A study by Wang et al. (1992) revealed that although both enantiomers had similar spermicidal effects in vitro, concentrations of free (-) gossypol decreased more than (+) gossypol when incubated with plasma proteins. Similar decreases were noted when rats were intravenously infused with purified enantiomers of gossypol. As a result, less (-)gossypol crossed the blood-testis barrier, and was barely detectable in rat testis fluid post infusion. Although this data suggests that the (-) enantiomer would be cleared more rapidly from the blood stream, this enantiomer has been identified as the most active in inhibiting biological activity of cellular proteins. Stereospecificity is a common phenomenon in biological compounds, due to the nature of protein binding (Voet and Voet, 1995).

Effects of gossypol on the female reproductive organs and embryos are even less well documented. In nonruminants, these effects are confounded with gossypol toxicity. Gossypol appears to disrupt estrous cycles, pregnancy and early embryo development in rats, hamsters, sows and humans (Randel et al., 1992).

To date there is no clear evidence of effects of gossypol on reproduction in ruminant females. In a study conducted by Gray et al. (1993) postpubertal beef heifers (n=6) and mature beef cows (n=8) were fed 0, 0.5, 2.5, 5.0, 10.0 and 20.0 g/d gossypol as both cottonseed meal and WCS to evaluate the effects of long term exposure to gossypol on metabolic and reproductive status. Treatment did not affect average daily gain in heifers or BCS. Heifers receiving the highest dose of gossypol had higher mean concentrations of luteinizing hormone (LH) compared to heifers in all other groups. In mature cows no difference was observed in magnitude of the preovulatory LH surge, luteal P_4 concentration, or concentrations of P_4 and estradiol in follicular fluid.

Heifers fed diet containing 0, 15 or 30% WCS for a 431 d period were of similar age and BW at onset of puberty (Colin Negrete et al., 1996). Response in LH surge following gonadotropin releasing hormone (GnRH) administration was not different among groups. However, other blood metabolites (blood urea N, creatinine, and alanine amino transferase, bilirubin) indicated possible liver and kidney impairment for heifers receiving WCS.

Gossypol also inhibited normal embryonic development and ovarian steroidogenesis *in-vitro*. Addition of gossypol to bovine embryos cultured *in-vitro* decreased the percentage of embryos that reached the blastocyst stage (Gu et al. 1990).

Bovine Somatotropin

Exogenous bovine somatotropin (bST) has been developed and approved for use in dairy cattle as an aid to enhance milk production. Somatotropin is a naturally occurring peptide hormone produced by the posterior pituitary. It has a wide range of effects, both direct and indirect, depending on the animal's physiological state. It stimulates synthesis and release of IGF-1 from the liver and possibly also from the ovary. Somatotropin receptors have been identified in ovarian tissue (Lucy, 1993a, Bilby and Lucy, 1997). Lactating Holsteins treated with bST have increased milk yields on average 10 to 15% in most trials and in commercial herds. Although stimulation of ovarian activity has been shown in response to bST treatment, pregnancy rates have been reported to be reduced or unaffected by bST (Bauman, 1992).

In vitro responses by ovarian granulosa and theca cell cultures to bST were examined in a study by Spicer and Stewart (1996). Basal production of estradiol by granulosa cells from small (1 to 5 mm) and large (>8 mm) follicles was not affected by bST treatment, however, 300 ng/ml bST inhibited estradiol production induced by follicle stimulating hormone (FSH) plus insulin in cells from both large and small follicles. Progesterone production was not affected by bST in these cultures. Thecal cell cultures had a greater than three-fold increase in androstenedione production in response to LH plus 3 to 30 ng/ml bST compared to those exposed to LH alone.

Ovarian follicular dynamics and circulating hormones were monitored in beef heifers receiving 4 x daily injections of carrier or 3.13, 6.25, 12.5, or 25.0 mg/d doses of bST (Gong et al., 1997). No effects of treatment were noted on circulating concentrations

of FSH, LH or P_4, nor was there an effect on number of follicles greater than 5 mm in size. Number of small (< 5 mm) follicles increased in response to bST in heifers which also showed increased IGF-1 concentrations, suggesting that bST stimulation of ovarian activity is mediated by IGF 1

Ewes injected with bST had increased concentrations of plasma FSH following immunization against inhibin, a protein produced by dominant follicles which promotes production of IGF 1 binding proteins, rendering them unable to stimulate growth of subordinate follicles (Tannetta et al., 1997) Inhibin immunized ewes treated with bST had a 2 2 fold greater ovulation rate and 1 8 fold greater litter size compared to ewes that did not receive bST

Injecting a sustained release form of bST (500 mg/14 d) shortened the interval between first and second follicular waves by 48 h in Holstein cows in a study by Kirby et al , (1997) Residual effects of bST on ovarian follicular dynamics was noted for three weeks following removal of bST treatment in this study Cows on continuous bST treatment for the 12 week treatment period tended to have greater P_4 concentrations than animals treated for six weeks with bST or saline and switched However, P_4 concentrations tended to be higher for cows receiving saline treatment throughout the experiment compared to bST treatments

Although bST has been shown to stimulate ovarian activity and, in some cases, to stimulate reproductive hormones it has also been reported to decrease pregnancy rates in high producing dairy cattle. This effect may be due to reduced body condition and prolonged NES

Supplemental fat has been studied as a potential means of mitigating the negative effects of bST treatment on reproduction. Cows in early lactation with or without 500 mg bST every 14 d received diets containing either no supplemental fat or 2.2 % of diet DM as Megalac® (Moallem et al., 1997). Cows on control diets plus bST ovulated for the first time postpartum after 30 DIM and had greater days open compared to other treatments, and pregnancy rates were reduced by bST. Cows receiving bST with or without Megalac had lower BW and BCS throughout the 140 d trial. Number of days to first postpartum ovulation was correlated with number of days to minimum BW. Days open were correlated with maximal BW loss, number of days to regain BW, number DIM to minimal BCS, and fat-corrected milk (FCM) production. Cows on the control treatment (no bST, no supplemental fat) regained BW sooner and exhibited estrus earlier than cows on other treatments.

Reproductive responses to bST may be dose dependant. In a multi-university study (Chalupa et al., 1996), cows (n=136) were administered 0, 10.3, 20.6, or 41.2 mg/d doses of bST for 38 weeks to examine the effects of bST on milk production and reproductive responses. Milk production increased quadratically in response to bST, with the highest production at the 20.6 mg/d dose. Although all cows gained BW during the trial, rate of BW gain decreased with increasing bST dose. Administration of 41.2 mg/d bST decreased pregnancy rate, and evaluation of common relative risk indicated that infertility was three times greater at this level of bST. Lower doses of bST, however, did not affect pregnancy rate in this study.

It is obvious that the effects of fat supplementation and bST on bovine metabolism reproduction and performance are highly complex and still not well understood Elaborating the myriad interactions of differing fat sources with other feed components, the rumen environment, endocrine function and tissue metabolism will no doubt occupy dairy scientists for many years to come.

Economic Analyses of Introducing Technological Change

In 1937, the United States government organized the US dairy industry into 41 regional regulatory agencies, termed Federal Milk Market Orders (FMMOs) The purpose of the FMMOs was to make their regions self-sufficient in milk and regulate milk prices paid by dairy processors to farmers (Bovard, 1989) Federal dairy policy guaranteed dairymen a minimum price for their milk. More recently dairymen in most states have organized into cooperatives which contract with processors for price and milk supply These cooperatives now control the majority of fluid class milk (Babb 1989)

Per cow milk production has increased markedly in the past 30 years due to improvements in genetics and feeding and technological change (Harris and Webb. 1990) Since 1979, milk supply has exceeded demand in the US, and because federal policies set a floor price for dairy products above world market price, exports have been limited (Bovard, 1989) Considerable criticism has been aimed at federal dairy policy resulting in numerous attempts to reduce US milk production, including the Dairy Diversion and Whole Herd Buyout programs of the 1980s (Howard and Shumway. 1988)

In spite of these efforts however milk output has continued to increase While the number of dairy farms has declined, size of dairies (head/farm) has increased,

particularly in the south and west Larger dairies are better able to exploit technological improvements Milking parlors, for example which increase not only per cow milk production but also number of cows milked per unit time, favor economies of scale (Weersink and Tauer, 1991)

Increasing input prices and declining prices for milk, cull cows and farm raised crops requires that dairies increase productivity or decrease costs. Productivity may be defined in terms of increased milk production per cow, per worker per acre or per dollar spent. Increasing productivity requires either increasing efficiency of production or allocation of resources, or technological change (Tauer, 1998)

Bravo-Ureta and Rieger (1990) compared a variety of methodologies for evaluating efficiency of dairy farms in the northeastern U S , including stochastic and linear models and least squares and maximum-likelihood statistical models. The four methods were highly correlated, and showed that technical efficiency was positively but weakly associated with farm size A stronger relationship was found between technical efficiency and return over variable cost per cow

Tauer (1998) conducted a study of 70 dairy farms in New York to study their productivity and its relationship to technological change. It was determined that average productivity increased 2.6 percent annually, primarily due to technological improvements. However, 25% of the farms in the study experienced productivity increases that were not sufficient to offset the decline in the ratio of output to input prices. Seventeen of the 70 farms experienced a decline or regression in technology, and thus accounted for much of

the lack of progress in productivity Technological change, in this report was not specified, but was assumed to have constant returns to scale.

Technology may be equipment, such as automatic milking machines, improved genetic stock, nutritional amendments, veterinary treatments such as vaccines, or hormonal treatments such as bST However technological change also comes at a cost.

A study conducted prior to FDA approval of bST, considered how bST adoption would affect farms of various sizes and the spatial distribution of dairying in the U S (Sellschopp and Kalter 1989) It was noted that the expected short run increase in national milk production if bST was widely adopted would result in a decrease in wholesale milk price The report predicted that under current federal support for dairy price, the share of milk production would shift to the west and southwest U S However in the absence of milk support pricing, total U S cow numbers would decline, and the share of milk production would increase in the Lake States, but decrease or remain unchanged in other regions

A number of methods have been employed to measure the economic health of dairies and the dairy industry in general Also a variety of tools have been developed to assist the dairyman to assess his/her own financial status, and help in decision making

As feed costs represent 50 to 70% of the input costs for dairies, a wide variety of programs have been developed to balance dairy cattle rations at minimum cost. These have been termed least-cost ration balancing programs, and many are used by dairymen and extensionists to help control feed costs However, it has been noted by Tauer that dairymen do not necessarily follow either cost minimizing or profit maximizing behavior

(Tauer, 1998). McCullough and DeLorenzo (1996) noted that producers base production decisions on numerous guidelines developed by scientists, but tempered by their personal intuition, and that, given changes in input and output prices and variation in management, generalities can rarely be applied

Additional regulations regarding the disposal of dairy manure and protection of water quality near dairies has led to the Okeechobee rule (Twichtman, 1990) named for the lake and surrounding land area where nutrient run off was alleged to cause eutrification of the lake Nutrient run off from dairy farms was considered to be a major contributor to the nutrification problems in the lake. As a result, farms throughout the state are now required to have nutrient disposal plans and to comply with Department of Environmental Protection standards for N and P as measured in test wells on the farm.

In order to assist dairyman to reduce and better manage manure nutrients, a model was developed to evaluate optimal nutrient recycling strategies for dairy farms in Florida (Henry et al , 1995) The model was designed to minimize feed and nutrient recycling costs by offering crops with high feeding value as well as their capacity to meet N and P uptake requirements. Varying average DMI of cattle in the model produced varying cropping strategies Forcing a minimum requirement for DMI on the model increased feed costs and required crop acreage for disposal of N compared to models where no minimum was imposed The model utilized sensitivity analysis to validate results

Reproduction and replacement strategies to improve productivity and profitability have received attention in recent years. Lehenbauer and Oltjen (1998) noted that culling decisions are an important influence on economic performance of dairies, and are affected

by financial considerations such as profit, cash flow and risk. As the salvage cost, defined as the difference between the price of a cull cow and replacement heifer, has increased, along with decreasing reproductive rates and increasing mill rates in herds in Florida and throughout the U S , tools to assist in insemination and culling decisions have been developed

McCullough and DeLorenzo (1996) used a stochastic dynamic model to evaluate the effects of price and management changes on optimal decisions for insemination or replacement of cows. A base run of the model using Florida data showed that average herd life on Florida dairies was 32 months For Florida based runs, the model recommended insemination on 98% of the time, reflecting the very low mean estrus detection (47%) and conception rates to AI (30%). Improvements in estrus detection and conception rate decreased the percentage of decisions to inseminate when input prices were held constant. Improvement in annual fertility therefore, increased the flexibility in decision making However, increasing pregnancy rate did not have as dramatic an effect on replacement decisions, as animals selected for culling were those that remained open late in lactation and were in the lowest production level. Nonetheless, pregnancy rate was the most dominant input influencing optimal replacement and insemination decisions

Component analysis of dairy economic performance and optimization models do not fully reflect the complex interactions that take place on and off the dairy and impact on the overall financial health of the individual dairy The complex responses of animals to nutrition, hormonal treatments, and other management decisions can have a profound impact on productivity, and hence, profitability of the dairy operation

CHAPTER 3
RESPONSE OF RUMEN BACTERIAL CULTURES TO FAT SOURCE AND METHOD OF INCORPORATION INTO FEEDSTUFFS IN VITRO

Introduction

Supplemental dietary fat has been included in rations for lactating dairy cattle for the last 100 years. However, the response to dietary fat is complex and still not fully understood. Animal responses vary with type and amount of fat, as well as other ingredients in the diet. Much of this response is dependent on reactions of the symbiotic bacteria which reside in the rumen.

Polyunsaturated fatty acids (PUFA) which predominate in vegetable oils and oil seeds are detrimental to fiber fermentation in the rumen, both *in vivo* and *in vitro* (Chalupa, 1984, 1986) and may be directly toxic to rumen cellulolytic bacteria (Mackie et al., 1991), possibly disrupting microbial membranes or interfering with binding of cellulolytic enzymes to substrate (Jenkins, 1993a). Changes in fiber fermentation appear to depend on the type of forage fed. Small particles may become more coated with fat and are less available for microbial enzyme attachment (Devendra and Lewis, 1974). Several studies have indicated that feeding alfalfa hay with or without other forages results in improved fiber digestibility, and hence greater milk production (Smith and Harris, 1992,

Smith et al , 1993, Adams et al , 1995) This may be the result of release of calcium from alfalfa, which may bind with free fatty acids (FFA) in the rumen to produce insoluble soaps, reducing the negative effects of FFA on bacteria (Palmquist and Jenkins, 1980)

Questions have been raised whether sequence of fat addition to concentrate or forage portions of the ration may affect ruminal, and, hence, animal response to dietary fat Drackley et al (1994) investigated effects of method of incorporation of fat on milk production and total tract digestibility of neutral detergent fiber (NDF). He noted no differences due to method of incorporation on total tract digestibility of NDF

Intestinal absorption of fatty acids is greatest for PUFA and least for saturated fat. However, a typical response to PUFA by rumen bacteria is to hydrogenate double bonds to produce saturated fatty acids which are less harmful (Mackie et al , 1991) and may be used for bacterial membrane synthesis (Bouchart et al 1990) Incomplete hydrogenation, which may result from high dietary fat levels or rapid passage rates of feed from the rumen, result in production of trans unsaturated fatty acids (TUFA) These TUFA have been shown to reduce milk fat production (Romo et al–1996) and may be involved in the development of human cardiovascular disease (Eog) 1996)

The objective of this research was to study the interactions among fat source rumen bacteria, and common feed ingredients *in vitro* as influenced by fat source and method of incorporation into feed Experiment 1 was a qualitative experiment to observe differences between calcium soap preparations in resulting fatty acid composition and resistance to biohydrogenation, preliminary to selecting a product for an *in vivo* study In Experiment 2 free fatty acids were extracted from solids as a means of comparing free

fatty acid release with patterns of biohydrogenation Also, liquid portion of fermentation flasks was extracted to account for poor recoveries of fatty acids from solids in trial runs The objectives of Experiments 3 and 4 were to determine if fat source and physical coating of forage or concentrate feeds with fat would effect NDF fermentation and biohydrogenation *in vitro*

Materials and Methods

Experiment 1. Production of Calcium Soaps and Stability *in Vitro*

Production of soaps

In a preliminary test, 100 g of poultry fat were added to a 1 L tared round bottom flask, followed by 500 ml 90% ethanol (450 ml absolute ethanol plus 50 ml deionized water) This was followed by 50 ml of 50% w/v aqueous NaOH (25 g NaOH crystals dissolved in 50 ml deionized water) The flask was then connected to a reflux apparatus and heated at 100°C for 1 hr An 85% w/v $CaCl_2$ solution was prepared by adding 109 g $CaCl_2$•$2H_2O$ to 100 ml water After refluxing was complete, the contents of the round bottom flask were poured into a 2 L Erlenmeyer flask and placed on low heat with stirring Contents were brought to 1 L by addition of absolute ethanol The $CaCl_2$ solution was added slowly to the Erlenmeyer flask..and a white precipitate formed. The contents of the flask were then poured into 500 ml plastic centrifuge bottles and centrifuged at 6000xg and 5°C for 20 min The supernatant was then removed and discarded The solid portion was freeze dried for 24 hr, yielding a fine white powder

A second batch of soap was prepared according to the method of Palmquist (personal communication) Four and one half kilograms poultry fat were poured into a 40

L steam kettle and heated to approximately 100°C Sodium hydroxide (700 g) dissolved in 15 L water was then added to the fat with stirring by a rotary stirring rod fashioned for the purpose and attached to an electric hand drill. Water was then added to bring total volume to 35 L Calcium chloride (1 kg 80%) was dissolved in water and added slowly to the heated, stirred solution. A yellowish solid formed and floated to the top. The contents of the kettle were then poured through a 2 mm wire mesh screen to recover soap The soap was then freeze dried for 48 hr

Soap extraction and chromatography

Soaps were extracted and methylated according to the method of Morrison and Smith (1964) Fifty milligrams of each sample (ethanol and water processes) were weighed into 15 ml leak-proof Teflon-lined screw cap tubes Two ml benzene, 1 ml 12% BF_3 in methanol, and 1 ml methanol were added in sequence to the tubes. The head space was flushed with N_2, the tubes capped tightly, vortexed for 30 s and placed in a boiling water bath for 1 hr The tubes were then cooled under running tap water for 5 min. The contents of each tube was washed with 2 ml deionized water and 2 ml hexane, vortexed and centrifuged for 5 min The bottom aqueous layer was removed with a Pasteur pipette and discarded, and the upper phase was washed a second time with 2 ml deionized water, vortexed, centrifuged. The upper phase was removed by pipette and loaded into Chromocal auto-injection vials (National Science Co , Lawrenceville, GA.)

Gas chromatography was performed on a Shimadzu GC-14A gas chromatograph (Shimadzu Corp , Japan) with a CPSil88 (Hewlett Packard, Palo Alto, CA) column using cod liver oil as an external standard. The injector and detector port temperatures were

300°C. The column was held at 155°C for 55 min then increased 2°C/min to a maximum of 225°C. Helium carrier gas was 1 75 kg/cc

In vitro fermentation study

Soaps were tested for inertness by in vitro rumen fermentation simulations according to the method of Moore and Mott (1974). Into each of eight 50 ml plastic tubes 0 5 g of soap (four ethanol samples and four water samples) were weighed with an equal amount of 1 mm glass beads (to break clumps)

Rumen fluid was collected from a fistulated lactating cow fed standard herd ration at the University of Florida Dairy Research Unit (Hague, FL) Fluid was strained through two layers of cheesecloth into a prewarmed thermos for transport to lab, and the cap tightly sealed. Fluid (100 ml) was strained again through cheesecloth into a 1 L Erlenmeyer flask containing 400 ml McDougall's Artificial Saliva (3 71 g Na_2HPO_4 anhydrous, 9 8 g $NaHCO_3$, 0 57 g KCl 0 47 g NaCl, 0 059 g $MgSO_4$ anhydrous/ L deionized water) warmed to 39°C in a water bath adjusted to pH 7 0 with CO_2 gas bubbled into the liquid buffer Defined nutrient solution (DNS) was added to the solution to provide substrates to maintain microbial populations.

To this mixture 1 ml $CaCl_2$ solution (4 g per 100 ml water) was added, and 30 ml of the buffered rumen fluid solution was added to 50 ml plastic centrifuge tubes, including four empty tubes which served as blanks. The head space of each tube was flushed with CO_2 and quickly stoppered with one way valved rubber stoppers to allow gas to escape The tubes were then incubated at 39°C for 48 hr

At the end of the first incubation period (Stage I), all tubes were removed and pH was tested with an Orion Research digital pH/millivolt meter 611 (Fisher Scientific, Atlanta, GA) Half of the tubes (two from each group) were removed from the rack, unstoppered and placed in refrigeration to stop fermentation The remaining tubes were treated with 30 ml HCl pepsin solution (20% v/v HCl and 5% w/v pepsin in 100 ml water), stoppered and returned to incubator for an additional 48 hr (Stage II)

Liquid phase of samples was poured into centrifuge tubes, and the solid portion washed with 10 ml petroleum ether to remove any fatty acids still associated with the solids according to the method of Sukhija and Palmquist (1990) Remaining ether was evaporated from solid portion by low heat under N_2 The solid residue was extracted, methylated, and chromatographed according to the methods described above.

Experiment 2. Comparison of Extraction Methods

Due to difficulties encountered in the prior experiment with recovery of fat from *in vitro* rumen samples these results were compared with an additional experiment using the method of Bligh and Dyer (1959) to extract fat from *in vitro* samples In addition, free fatty acids (FFA) were extracted using the method described by Reddy et al (1993) to correlate FFA release with biohydrogenation. A secondary objective of this experiment was to determine the number of time periods most appropriate to characterize the pattern of biohydrogenation

Rumen fluid was collected and *in vitro* fermentation carried out as described previously with the following alterations. Ninety ml buffered rumen fluid was added to 125 ml Erlenmeyer flasks containing 1 5 g DM, with a total of three flasks per

treatment/time period. Treatments included blank, control (35% corn silage, 35% alfalfa hay, and 30% hominy), corn oil, or poultry fat added as 3% of DM to replace hominy. Feed samples were collected from the University of Florida Dairy Research Unit, dried at 55°C for 48 hr and ground through a 1 mm screen in a Wiley Mill. Blank samples contained only buffered rumen fluid and DNS. Time periods selected were 0, 12, 24, 48, and 96 h. Stage II acid/pepsin incubation was carried out during the final time period. Three flasks per treatment were acidified immediately with 30 ml 20% (v/v) HCl, and stored at 4°C. These samples constituted 0 h incubation. All samples were run in duplicate.

At the end of each incubation period, all flasks were removed and pH was tested with an Orion Research digital pH/millivolt meter 611 (Fischer Scientific, Atlanta, GA). Three flasks per treatment group were removed, acidified and stored at 4°C.

Extraction and gas chromatography

Contents of all flasks were poured into 250 ml plastic centrifuge bottles and spun at 5000 x g for 20 min. The contents were vacuum filtered through Whatman No. 41 filter paper. Liquid portion was returned to the bottles and stored at 4°C. The liquid phase was poured into 250 ml separatory funnels and extracted with benzene according to the method of Bitman et al (1984).

Solid residue was divided into two portions, transferred to 50 ml plastic centrifuge tubes, frozen at -20°C overnight, and freeze dried for 24 h. One half of the solid residue from each flask was extracted according to the method of Bligh and Dyer (1959) to determine total fatty acids. Fifteen ml chloroform:methanol (1:2) was added to the residue

and blended for 2 min using a Polytron homogenizer The homogenate was vacuum filtered through Whatman No. 1 filter paper on a No 3 Buchner funnel with light suction. Filtrate was transferred to a 50 ml screw cap tube The remaining residue and paper were extracted a second time with 5 ml $CHCl_3$, refiltered through the Buchner funnel, and the filtrates combined Five ml of a 0 88% w/v KCl solution was added to each extract, the tubes sealed, vortexed, and centrifuged to separate layers The lower chloroform phase containing the lipid fraction was removed and dried under N_2 in a 50°C warm water bath.

Remaining solid portions were extracted according to the method described by Reddy et al (1993) to separate FFA from triglycerides to determine extent of lipolysis. Fractions were extracted with a 1 1 mixture of ether:acetone (10 ml) and washed twice with 2 ml 0 05 N K_2CO_2 solution. The bottom aqueous phase was removed by Pasteur pipette and acidified with 24 ug 75% H_2SO_4 to pH 2.5 and extracted with 5 ml hexane Hexane phase was removed, washed with water and dried under N^2 in a warm water bath at 45°C The remaining oily residues from the solid and liquid fractions were methylated according to the method of Morrison and Smith (1964). Nonodecanoic acid (C19:0) was added to each sample at a rate of 0.2 mg/ml benzene as an internal standard Chromatography was performed as described previously

Statistical analysis

Data were analyzed according to the general linear models procedure of SAS (1987) The model may be expressed as.

$$Y=\mu+T+H+T*H+e$$

where Y=NDF or individual fatty acid

μ=overall mean

T=treatment

H=time in hours

and e=residual error

Experiment 3. Effects of Fat Source and Method of Incorporation into Feed Components I. Inocula Unconditioned to Fat

Following these investigations, another experiment was performed to determine the effects of fat source and method of incorporation on biohydrogenation patterns and NDF fermentation in a 7 x 3 x 4 factorial arrangement. Six fat sources, whole cottonseed (WCS), poultry fat (PF), tallow (TALL), calcium soaps of poultry fat (CaPF), fish oil (FO), and corn oil (CO). Each source was mixed with either corn silage (CS), alfalfa (AH), or hominy (HOM) first before being combined with the other ingredients. Feed samples were collected and prepared as described previously. Samples for NDF and fatty acid analyses were prepared and incubated separately. All samples were run in duplicate.

Because of high basal levels of 18-carbon fatty acids in the blank samples from the previous experiment, it was decided that a further test was necessary using a donor animal that had been on a low fat diet. The second animal was a non lactating adult cow which had been fed on a diet consisting of bermudagrass hay and a soybean meal-based pellet with no additional fat. Rumen fluid was collected and prepared as described previously.

Neutral detergent fiber was analyzed according to the method of Goering and Van Soest (1970) with the following modifications. Entire contents of flasks were poured into 600 ml Berzelius beakers and flasks rinsed with NDF solution. Neutral detergent solution

(150 ml) was added to each beaker and beakers were placed on a reflux apparatus and allowed to come to a boil. After boiling for 5 min, 2 ml of α-amylase (Sigma-Aldrich Corp, St. Louis, MO) was added to each beaker, and the beakers returned to reflux unit and allowed to boil for 1 h. Beakers were removed from heat, and an additional 2 ml α amylase added, and beakers were allowed to stand for 30 sec. Contents of beakers were filtered through ashed, preweighed ceramic porcelain crucibles containing glass wool. Remaining residue was rinsed with boiling water followed by acetone, and dried in a forced air oven at 100°C overnight. Crucibles were weighed and ashed in a muffle furnace at 500°C for 6 h and reweighed for organic matter determination.

For fatty acid analysis, solid residues were lyophilized and subjected to the Bligh and Dyer extraction method (1959) and methylated according to the method of Morrison and Smith (1964) with 19:0 as an internal standard. Samples were run on a Hewlett Packard 5890 gas chromatograph with an SP 2340 (Sigma Aldrich, Inc., St. Luis, MO) 30 m column. Injector and detector ports were set at 225 and 245°C respectively. Initial oven temperature was set at 140°C and increased 4°C per minute to a maximum of 240°C and held for 15 min.

Statistical analysis

Data from NDF and fatty acid analyses were analyzed by the general linear models procedure of SAS (1987). The model may be expressed as:

$$Y=\mu+T+H+T*H+e$$

where Y=NDF or individual fatty acid

μ=overall mean

T=treatment

H=time in hours

and e=residual error

Orthogonal contrasts were control vs fat, hominy vs. roughages, alfalfa vs corn silage, animal vs. vegetable fats, fish oil vs. other animal fats, 0 h vs. other periods, 0 and 12 hrs vs. 24 and 48 h, and 0, 12 and 24 hrs vs 48 hrs for NDF For fatty acid analyses, conducted only on 0, 12 and 24h samples, contrasts for time were 0 vs 12 and 24h, and 12 vs. 24h An example ANOVA and orthogonal contrasts for 18:2n6 are presented in Table 3-1

Experiment 4. Effects of Fat Source and Method of Incorporation into Feed Components II. Inocula Previously Conditioned to Fat

To confirm results of NDF analysis in the previous experiment, an additional experiment was performed using a cow consuming a 15% WCS ration at the Dairy Research Unit as a donor Fat sources for this experiment were WCS, TALL and FO, at either 3 or 6% of DM and incorporated into feed mix as described above Samples and controls (no added fat) were incubated for 48 h for NDF analysis as described above. Rumen fluid was collected on two consecutive days and all samples were carried out in duplicate across two fluid collections.

Statistical analysis

Data were analyzed by the general linear models procedure of SAS (1987) The model included fat source fat level (3 or 6% of DM), source x level, method of incorporation, source x method, level x method, and source x level x method.

Significance was declared at $P<0.05$. Day of rumen fluid collection was not significant. Additional three and four-way interactions involving day of collection were dropped from the final model as they were not significant.

Results

Experiment 1

Fatty acid composition of soaps are presented in Table 3-2. Linoleic acid concentration of water and ethanol process soaps was 17.8 and 18.5% of total fatty acids. The CPSil-88 column is designed to detect trans fatty acids, which often result from partial biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen. No *trans* 18:1 fatty acids were detected in the blank rumen fluid samples after either stage of incubation.

No *trans* 18:1 was detected in the washes of either ethanol or water process soaps after stage I incubation. However, increases in 18:2ω6 were detected in post-fermentation stage soap samples compared with soaps that had not been incubated. These increases may be accounted for by contributions from microbial cells, since blanks contained between 4 and 6% 18:2ω6, and the increase in 18:2ω6 in post fermentation soap samples was approximately 5%.

Water process soap samples resulted in an average 2.5% increase in *trans* 18:1 after stage II incubation (acid/pepsin stage) compared to original soap, indicating the amount of biohydrogenation, as 18:2ω6 levels decreased comparably. Increases in *trans*

18:1 in ethanol process samples ranged from 6 to 40% There were also increases in 18:0 in both treated soap samples after Stage II In the ethanol process soaps as much as 30% increase in 18:0 was noted Increases in 18:0 in the water process samples were less. The average concentration of 18:2ω6 remaining in the ethanol process soaps after Stage II was 13.52%, but this varied between runs, the range was from 10.98 to 15.93% Less variation was noted in the water process samples, ranging from 16.70 to 17.16%.

Differences between the two processes may be due to the different physical qualities of the two products Ethanol soaps were dry and powdery, and clumps were easily broken. Palmquist (personal communication) noted in his experiments that finely powdered soaps were up to 90% biohydrogenated, while coarser granules were only 40 to 50% biohydrogenated Water process samples were sticky and tended to form larger clumps more resistant to crumbling The ethanol samples may have provided more surface area for bacterial attachment, resulting in greater hydrolysis of triglyceride bonds and thus greater hydrogenation. It should be noted that no attempt was made in these experiments to standardize particle size

Experiment 2

Least squares means for recovery of fatty acids from total solids extraction, FFA extraction, and extraction of rumen fluid are expressed in Tables 3-3 through 3-5, respectively The predominant fatty acids in total extracts across treatments were 16:0, 18:0, and 18:1ω9 Palmitic acid was higher in PF samples compared to CO (3.58 vs. 2.83 mg, P=0.0296) and lowest in blanks (1.15 mg, P=0.0024) Stearic acid and 18:1ω9 were also highest in fat treatments, but did not differ between PF and CO Content of *trans* 18:1

was also highest in fat treatments, and CO samples tended to contain more *trans* 18:1 than PF (2.65 vs. 2.02 mg; $P=0.09$) (Table 3-3).

Differences in FFA were noted only for blanks vs. other treatments (Table 3-4). Free 18:0 was highest in blank samples compared to other treatments (1.58% vs. 0.68, 0.50, and 0.61 for control, PF and CO, respectively; $P=0.0001$). In general, fatty acids in rumen fluid were low, with the exception of 16:0, but still constituted a substantial portion of total fatty acids of *in vitro* samples (Table 3-5). Mean quantities of 18:0, 18:1ω9, and 18:2ω6 in rumen fluid were 0.26, 0.32, and 0.13, respectively. Palmitic acid was lowest in blanks compared to other treatments (0.69 vs. 1.81, 1.93, 1.93 for control, PF and CO, respectively, $P=0.0001$).

Although total fat in blanks was considerably lower than for control and fat added groups, amounts of free 18-carbon fatty acids in blanks and controls were at points as high as those of the fat-treated samples (Table 3-4). This varied with time and fatty acid. At 12 h, amount of 18:0 and *trans* 18:1 in blanks were highest (Table 3-6), with the peak in 18:0 equivalent to both corn oil (Table 3-9) and poultry fat means (Table 3-8), and higher than that of the control samples. Content of 18:1ω9 and 18:2ω6 peaked in blanks at 24 h, with 18:1ω9 content equivalent to that of controls, and 18:2ω6 higher than control and equivalent to that of poultry fat.

Possible explanations for this phenomenon are not apparent, but it is possible that previous exposure to dietary fat in the donor animal from which fluid was obtained resulted in higher fat content in microbial cells. Bauchart et al., (1990) noted as much as 150% increase in microbial lipid content when cells were incubated with fat. The donor

cow for this experiment had been maintained on the herd lactation ration, which includes approximately 9% WCS. This high level of fat in the diet may have resulted in artificially high levels of fat in the microorganisms, and thus influenced the results of this trial. Although samples were filtered to remove bacteria, some cells and feed particles may have passed through the filtering material.

Pattern of change in 18-carbon fatty acids is depicted in Figure 3-1. Linoleic acid tended to decline throughout the first 24 h (P=0.0778), while 18:1ω9 increased to a peak at 12 h incubation before also declining sharply (P=0.0249). Stearic acid and *trans* 18:1 began increasing at 12 h, with *trans* 18:1 reaching peak at 24 h before beginning to decrease, while 18:0 continued to increase through the end of the incubation period. Since most of the 18-carbon fatty acids could be accounted for mathematically in one isoform or another, the pattern suggests a time course for biohydrogenation.

The pattern of biohydrogenation was similar to that seen in catalytic hydrogenation of PUFA with Ni (O'Keefe, personal communication). The n-6 double bond of linoleic acid is first hydrogenated to yield 18:1ω9. The n-9 bond is then isomerized to a *trans* configuration before final hydrogenation of the bond to form 18:0. This pattern was clearest in the samples containing corn oil. However, a similar pattern was also evident in PF samples, although at a lower magnitude due to the lower concentration of 18:2ω6 in PF compared to CO.

Hydrolysis of triglycerides to FFA varied with time and fatty acid (Tables 3-6 through 3-9). Release of free 18-carbon fatty acids was highest among blanks at 12 h, whereupon it declined to a minimum at 24 h and then increased again. Stearic acid release

from other treatments varied less dramatically with time, and was lowest for control and poultry fat samples.

The quantities of *trans* 18:1 found free over time were more highly variable among treatments. At 12 h, free *trans* 18:1 was highest among controls, while at 48 h, free *trans* 18:1 was highest in corn oil samples. Free *trans* 18:1 was lowest in poultry fat compared to other treatments for all time periods.

In contrast, free 18:1ω9 was highest in poultry fat samples at 12 h (Table 3-8), although they had decreased to below 0 h levels by 24 h. Control samples (Table 3-7) peaked lower and later than poultry fat, but showed the same steep decline by 48 h. Surprisingly, levels of 18:1ω9 in corn oil (Table 3-9) were lower than blanks for all time periods, and amplitude of the peaks were considerably smaller than for control and poultry fat. Free 18:2ω6 tended to decline with time for corn oil, while control and poultry fat samples showed increased 18:2ω6 at 12 h, followed by decreases in that fatty acid.

For most treatments, amounts of free 18-carbon fatty acids tended to increase after acid/pepsin incubation. Reddy et al. (1993) also noted fluctuations in the amounts of free fatty acids of *in vitro* rumen fluid samples. It has been suggested by Palmquist and Kinsey (1994) that fatty acids may be reesterified in the rumen. Bauchart et al. (1990) indicated that substantial amounts of fat may be taken up by rumen bacteria and stored in vesicles inside the cells. It is likely that reesterification of fatty acids takes place inside the bacterial cell in order to store fat, and that fatty acids are released by acid hydrolysis

Experiment 3

Results of the fermentation/NDF experiment are depicted in Table 3-10. Mean NDF percentage of mixed feed samples was 35.06 %. Surprisingly, mean NDF residue was higher in control (no fat) samples compared to fat-treated samples (P=0.0157), but no differences were noted among fat treatments, nor was method of incorporation into feed samples significant. Interactions for treatment x time were significant for 15:0 (P=0.0001), 15:1ω6 (P=0.0001), 17:1ω8 (P=0.0025), 20:5ω3 (P=0.0006) and 22:6ω3 (P=0.0015), but not for 18-carbon fatty acids.

Output of statistical analyses of fatty acid data are presented in the Appendix. Fatty acid composition of fat sources and mixed feed ingredients are in Table 3-11. Pattern of biohydrogenation of 18-carbon fatty acids was similar regardless of fat type and method of incorporation. Patterns of change in 18 carbon fatty acids were pooled and are depicted in Figure 3-2. On average, 18:0 increased throughout the 24 h incubation from 3.60 to 13.89% (P=0.001). Oleic acid increased during the first 12 h from 18.34 to 25.96% (P=0.001) before leveling off at 26.56% by 24h. *Trans* 18:1 increased from 12h to 24h (0.16 to 0.52%, p=0.0071) while *trans* 18:2 decreased during this time period (0.19 to 0.05%, P=0.0001). Linoleic acid tended to decrease during the first 12h period (28.50 to 27.52%, P=0.0645) and then decreased more sharply from 12 to 24 h (23.42%, P=0.0320). Linolenic acid (18:3w3) decreased throughout the 24 h incubation from 2.21 to 1.11%(P=0.0001). High levels of 18:1ω9 were maintained even while 18:0 increased since 18:2ω6 and 18:3ω3 were converted to 18:1ω9 more rapidly than 18:1ω9 was converted to 18:0.

Both type of fat and method of inclusion were significant for mean 18:0. In general, animal fats mixed first with hominy before incorporating into the total feed mixture resulted in lower levels of 18:0 compared to mixing with roughages (7.38 vs. 8.91 %) while vegetable sources, principally corn oil, yielded higher 18:0 levels when mixed first with hominy (8.96 vs. 7.53 %; $P=0.0356$). Response in 18:0 production varied amongst animal fat sources when mixed with either CS or AH. Menhaden fish oil mixed first with AH resulted in greater 18:0 production compared to CS (6.07 vs. 9.77%) while TALL and CaPF yielded more 18:0 when mixed first with CS (12.63 and 9.11 vs. 9.18 and 8.59%, respectively, $P=0.0073$). Poultry fat performed similarly to FO. Corn oil mixed first with HOM resulted in higher 18:0 production compared to mixing first with roughage (9.92 vs. 6.23), while WCS mixed first with HOM yielded less 18:0 (8.00 vs 8.84%, $P=0.049$).

Interactions between fat source and method of incorporation were not significant for other fatty acids. Interactions of treatment with time were significant only for 15:0 ($P=0.0005$) and 15:1ω6 ($P=0.0031$), which decreased over time in fat treatments compared to controls. These fatty acids are of microbial origin and may reflect either a decrease in microbial growth or the substitution of *de novo* synthesized microbial lipids with fatty acids provided by supplementation.

Experiment 4

Results of NDF analysis are in Table 3-12. No differences were detected due to fat source, level of fat, or method of incorporation in NDF disappearance from samples incubated with rumen fluid preconditioned to dietary fat. Mean NDF disappearance was

51.21%. Standard error of the mean was 1.122. Calculations show that 32 replicates per parameter would have been required to conclude that differences among treatments were significant.

Discussion

Soaps prepared with ethanol contained less 18:2ω6 per gram than water process soaps and appear to be more susceptible to microbial attack than are water process soaps. As much as 40% of unsaturated fatty acids in ethanol process soaps were biohydrogenated during *in vitro* tests, while only approximately 2.5% of water process fatty acid soaps were biohydrogenated. This was most likely due to the differences in consistency between the two soaps. Ethanol soaps were dry and powdery, while water process soaps were sticky and formed comparatively large, coarse clumps. As a result, as little as 11% of 18:2ω6 may be available to the small intestine if ethanol process is used, while 16 to 17% would be made available by the water process soap. However, the consistency of the water process soap and its rather strong odor may pose problems in feeding. It may be possible to mix the soap with a dry, palatable carrier such as corn meal to make it more practical for inclusion in dairy cattle diets.

The pH of the solutions after stage 1 ranged from 6.11 to 6.56, near normal rumen pH. There remains some question as to whether soaps would dissociate in this range and possibly reform. Sukhija and Palmquist (1990) noted that the pKa of soaps high in PUFA was higher than that of palm oil fatty acids, making them more likely to dissociate in the

rumen. While rumen pH fluctuates, particularly after a grain meal is consumed, the buffered *in vitro* solution is more stable.

Regardless of source of inoculum, NDF disappearance was unaffected by fat treatment or method of incorporation. The high level of AH in these experiments may have limited detrimental effects of fat on fiber fermentation. Smith et al., (1993) noted increased total tract NDF digestibility of fat treated diets with AH at 12.5% of DM. Alfalfa hay comprised between 30 and 35% of DM in samples containing fat in these experiments. Lack of an effect of method of incorporation on NDF digestibility *in vitro* is supported by findings *in vivo* by Drackley et al., (1994) who found no differences in total tract digestibility of NDF due to method of fat incorporation into the ration.

The recovery of substantial amounts of long chain fatty acids in rumen fluid is in contrast to the findings of Sukhija and Palmquist (1990). These researchers stated that long chain fatty acids, due to hydrophobic interactions, would not be found in significant amounts in rumen fluid. Filtration method or filter paper may have permitted passage of bacteria and very small particles into the fluid filtrate in the current experiment.

Fatty acid hydrogenation patterns differed depending on fat source and method of incorporation for odd-chain and 20-carbon fatty acids. However, the biological significance of this finding is unknown.

Table 3-1. Example ANOVA for 18:2ω6 (Experiment 3)

Source	df	SS	MS	*F*	*P* =
Treatment.	19	3338.31	175 70	3 77	0 0001
Control (no fat)					
Corn oil/Hominy					
Corn oil/Alfalfa					
Corn oil/Corn Silage					
Tallow/Hominy					
Tallow/Alfalfa					
Tallow/Corn Silage					
Poultry fat/Hominy					
Poultry fat/Alfalfa					
Poultry fat/Corn Silage					
Calcium soap/Hominy					
Calcium soap/Alfalfa					
Calcium soap/Corn Silage					
WCS/Hominy					
WCS/Alfalfa					
WCS/Corn Silage					
Fish meal/Hominy					
Fish meal/Alfalfa					
Fish meal/Corn Silage					
Time (linear)	1	316 60	316 60	6 80	0 0104
Time (quadratic)	1	0 0012	0 0012	0 00	0 9960
Treatment x Time (linear)	19	446 20	23 48	0.50	0 9556
Treatment x Time (quadratic)	19	560 70	29.51	0 63	0 8726
Residual (R)	108	5027.57	46 55		

Table 3-1 -- contd.

Orthogonal Contrasts	df	SS	MS	F	P =
Treatment					
Control v Fat	1	145 53	145.53	3.13	0 0799
Hominy v Roughage	1	89 38	89 38	1.92	0 1687
CS v AH	1	24 74	24 74	0.53	0 4676
Vegetable Fat v Animal Fat	1	2070 91	2070 91	44 49	0 0001
Fish Oil v Other Animal Fats	1	212 44	212 44	4.56	0 0349
Ca PF v Tallow, PF	1	42 26	42.26	0 91	0.3428
Tallow v PF	1	20 31	20 31	0 44	0 5104
(Hominy v Roughage) x (Vegetable Fat v Animal Fat)	1	250 70	250 70	5 39	0 0222
(Hominy v Roughage) x (Corn Oil v WCS)	1	109 25	109.25	2.35	0 1285
(Hominy v Roughage) x (Fish Oil v Other Animal Fats)	1	19 22	19.22	0 41	0.5219
(Hominy v Roughage) x (Tallow v PF)	1	37 33	37 33	0 80	0 3725
(CS v AH) x (Vegetable Fat v Animal Fat)	1	35 35	35 35	0 76	0 3855
(CS v AH) x (Corn Oil v WCS)	1	0 69	0 69	0 01	0 9034
(CS v AH) x (Fish Oil v Other Animal Fats)	1	6.11	6 11	0 13	0 7179
(CS v AH) x (Tallow v PF)	1	5 73	5 73	0 12	0 7264
Time (linear)	1	316 60	316 60	6 80	0.0104
Time (quadratic)	1	0 0012	0 0012	0 00	0 9960

Table 3-2. Composition of calcium soaps of poultry fat and fermentation and digestion products (% of total fatty acids)

	Untreated Soaps		Stage I - Fermentation		Stage II - Acid/Pepsin	
Fatty Acid	Ethanol	Water	Ethanol	Water	Ethanol	Water
14:0	0.81	0.75	1.59	0.95	1.00	0.88
14:1ω5	0.20	0.23	0.00	0.35	0.34	0.32
16:0	23.98	23.24	21.32	22.22	24.47	22.40
16:1ω7	6.33	6.79	6.86	6.66	5.35	6.71
18:0	5.71	5.46	4.53	5.70	8.36	5.56
18:1ω9	38.10	37.95	42.51	38.34	34.77	38.30
*trans*18:1	1.95	1.86	2.52	2.24	4.74	2.24
*trans*18:2	0.19	0.66	0.00	0.56	0.65	0.60
18:2ω6	17.86	18.50	13.66	17.16	13.52	16.91
18:3ω3	0.66	0.79	0.00	0.76	0.35	0.76
22:6ω3	0.16	0.06	0.28	0.06	0.00	0.05

Table 3-3 Experiment 2. least squares means of total fatty acids recovered from solids (in milligrams[a]).

	Treatment				Orthogonal Contrasts (*Pr*>)		
FA	Blank	Control	Poultry Fat	Corn Oil	Blank v others	Control v. fat	Corn Oil v Poultry Fat
14 0	0 0456	0 1049	0 1536	0 1423	0 0001	0 0079	ns
14 1ω5	0.0392	0 1421	0 1726	0 1880	0 0001	0 0719	ns
15 0	0 0300	0 0889	0 1011	0 0995	0 0001	ns	ns
16 0	1 1526	2.2779	3 5765	2 8325	0 0001	0 0024	0 0296
16 1ω7	0 0344	0 0603	0.2517	0 1225	0 0001	0 0001	0 0001
17 0	0.0238	0 0698	0 1040	0 0833	0 0001	0 0296	0 0984
18 0	2.2043	3.6841	4 1603	4 3730	0 0001	ns	ns
*trans*18 1	0 0467	1 9563	2 0225	2 6478	0 0001	ns	0 0931
18 1ω9	0 5758	1 5771	3.4099	3 3967	0 0035	0 0166	ns
18 2ω6	0 5555	1 1028	1 9707	2 3117	0 004	ns	ns

[a] milligrams calculated according to peak area of internal standard by the equation (mg standard/area standard) * mg unknown/area unknown

Table 3-4 Experiment 2. least squares means of free fatty acids recovered from solids (in milligrams).

	Treatment				Orthogonal Contrasts ($P=$)		
FA	Blank	Control	Poultry Fat	Corn Oil	Blank v others	Control v fat	Corn Oil v Poultry Fat
14:0	0.0847	0.0339	0.0491	0.0389	0.0022	ns	ns
14:1ω5	0.0441	0.0765	0.0713	0.0787	0.0534	ns	ns
15:0	0.0261	0.0077	0.0034	0.0047	0.0006	ns	ns
16:0	0.8756	0.4230	0.3978	0.4411	0.0001	ns	ns
16:1ω7	0.0757	0.0335	0.0458	0.0390	0.0002	ns	ns
17:0	0.0187	0.0025	0.0027	0.0065	0.0042	ns	ns
18:0	1.5805	0.6831	0.5006	0.6057	0.0001	ns	ns
trans18:1	0.3821	0.3945	0.2516	0.3112	ns	ns	ns
18:1ω9	0.6924	0.4927	0.4936	0.5118	0.0065	ns	ns
18:2ω6	0.2421	0.1455	0.1411	0.2185	ns	ns	ns

Table 3-5 Experiment 2 least squares means of fatty acids recovered from rumen fluid (in milligrams)

	Treatment				Orthogonal Contrasts		
FA	Blank	Control	Poultry Fat	Corn Oil	Blank v others	Control v fat	Corn Oil v Poultry Fat
14 0	0 2151	0 2014	0 1945	0.2138	ns	ns	ns
14 1ω5	0 0303	0 0438	0 0330	0 0666	ns	ns	ns
16 0	0 6889	1 8105	1 9276	1 9228	0 0001	ns	ns
16 1ω7	0 0103	0 0442	0 0129	0 0121	ns	ns	ns
17·0	0 0000	0 0045	0 0000	0 0000	ns	ns	ns
18 0	0 2044	0 3108	0 2683	0 2541	ns	ns	ns
trans18 1	0 0506	0 1112	0 0652	0 0676	ns	0 0905	ns
18 1ω9	0.2564	0 3374	0 3335	0 3433	0 0216	ns	ns
18 2ω6	0.2033	1.3771	1.2007	1.2289	ns	ns	ns

Table 3.6 Experiment 2: free fatty acids recovered from solid portion of blanks (in milligrams)

	Time (hrs)					
FA	0	12	24	48	96	*P* value
14:0	0.059	0.089	0.115	0.056	0.105	ns
14:1ω5	0.035	0.084	0.028	0.060	0.014	ns
15:0	0.000	0.047	0.000	0.028	0.056	ns
16:0	0.845	1.087	0.420	0.915	1.111	ns
16:1ω7	0.073	0.101	0.044	0.061	0.100	0.0461
17:0	0.014	0.013	0.011	0.020	0.036	ns
18:0	1.343	2.060	0.560	1.595	2.341	ns
trans18:1	0.224	0.542	0.162	0.560	0.422	ns
18:1ω9	0.745	0.774	0.542	0.770	0.630	0.0249
18:2ω6	0.419	0.204	0.181	0.291	0.116	0.0778

Table 3-7. Experiment 2 free fatty acids recovered from solid portion of control (in milligrams)

	Time (hrs)					
FA	0	12	24	48	96	*P* value
14:0	0.024	0.041	0.053	0.035	0.016	ns
14:1ω5	0.049	0.093	0.130	0.057	0.054	ns
15:0	0.022	0.000	0.000	0.000	0.014	ns
16:0	0.559	0.537	0.441	0.161	1.417	ns
16:1ω7	0.032	0.053	0.058	0.013	0.012	0.0461
17:0	0.013	0.000	0.000	0.000	0.000	ns
18:0	1.051	0.666	0.728	0.206	0.765	ns
trans18:1	0.379	0.626	0.560	0.095	0.313	ns
18:1ω9	0.440	0.729	0.771	0.271	0.253	0.0249
18:2ω6	0.162	0.312	0.172	0.012	0.070	0.0778

Table 3-8. Experiment 2. Free fatty acids recovered from solid portion of PF treated samples (in milligrams).

	Time (hrs)					
FA	0	12	24	48	96	*P* value
14:0	0.050	0.076	0.027	0.036	0.056	ns
14:1ω5	0.051	0.073	0.089	0.082	0.062	ns
15:0	0.017	0.000	0.000	0.000	0.000	ns
16:0	0.500	0.555	0.228	0.198	0.508	ns
16:1ω7	0.049	0.094	0.023	0.030	0.033	0.0461
17:0	0.014	0.000	0.000	0.000	0.000	ns
18:0	0.889	0.486	0.272	0.254	0.603	ns
trans18:1	0.286	0.353	0.172	0.106	0.341	ns
18:1ω9	0.420	0.892	0.342	0.347	0.468	0.0249
18:2ω6	0.079	0.270	0.073	0.064	0.220	0.0778

Table 3-9 Experiment 2 free fatty acids recovered from solid portion of CO treated samples (in milligrams)

	Time (hrs)					
FA	0	12	24	48	96	*P* value
14:0	0.026	0.041	0.026	0.069	0.033	ns
14:1ω5	0.025	0.058	0.087	0.078	0.145	ns
15:0	0.000	0.000	0.000	0.024	0.000	ns
16:0	0.236	0.601	0.404	0.764	0.201	ns
16:1ω7	0.025	0.042	0.021	0.064	0.043	0.0461
17:0	0.017	0.000	0.000	0.015	0.000	ns
18:0	0.192	0.906	0.483	1.253	0.195	ns
trans18:1	0.062	0.423	0.352	0.673	0.045	ns
18:1ω9	0.383	0.590	0.449	0.677	0.460	0.0249
18:2ω6	0.304	0.300	0.170	0.176	0.143	0.0778

Table 3-10 Experiment 3: effects of type of fat and method of incorporation on percentage NDF remaining after incubation *in vitro*

Treatment		Time (hrs)			
		0	12	24	48
Control		35.24	32.55	26.12	21.53
Corn Oil	hominy	36.39	33.30	15.67	18.36
	alfalfa hay	35.98	32.93	27.51	17.80
	corn silage	37.00	32.20	25.93	18.12
Tallow	hominy	34.87	31.95	25.94	18.66
	alfalfa hay	34.10	30.67	26.01	17.00
	corn silage	35.11	31.91	24.99	17.94
Poultry Fat	hominy	35.43	30.75	25.60	18.63
	alfalfa hay	36.95	31.68	25.43	16.88
	corn silage	36.07	30.94	26.25	17.54
Ca-PF	hominy	34.23	29.50	25.14	17.90
	alfalfa hay	34.06	31.63	24.26	19.49
	corn silage	33.22	29.88	25.67	19.54

Table 3-10—contd.

		Time (hrs)			
		0	12	24	48
Fish Oil	hominy	34 04	34 01	25 60	18.25
	alfalfa hay	34 46	30 60	26 11	16 63
	corn silage	33 83	32 05	26.31	18 65
WCS	hominy	35.30	29 98	27 04	17 17
	alfalfa hay	35 16	31 47	23 88	17 43
	corn silage	34 64	30 00	24 24	16 10

Table 3-11 Fatty acid composition of fat and feed stocks (%)

	Corn Oil	WCS	Tallow	Poultry Fat	Ca-PF	Fish Oil	Feed Mix
12:0	0.00	0.00	0.08	0.05	0.00	0.00	0.00
14:0	0.03	0.61	2.18	0.70	0.76	8.05	3.24
14:1ω5	0.00	0.02	0.32	0.22	0.39	0.09	0.18
16:0	10.81	22.18	23.76	23.26	23.64	21.15	20.36
16:1ω7	0.09	0.48	2.89	6.59	6.91	9.69	6.68
18:0	1.88	2.09	13.42	5.59	5.56	4.57	6.40
18:1ω9	25.91	14.66	41.51	39.89	38.67	7.62	21.07
*trans*18:1	0.00	0.06	2.03	1.86	1.89	0.00	0.75
18:2ω6	58.85	0.00	0.00	17.65	18.85	3.85	32.13
18:3ω3	0.04	0.21	0.41	0.77	0.40	1.30	5.14
20:4ω6	0.00	0.00	0.00	0.27	0.32	1.41	0.00
20:5ω3	0.00	0.00	0.00	0.00	0.00	9.77	0.00
22:6ω3	0.00	0.00	0.00	0.14	0.00	8.52	0.22

Table 3-12. Least squares means of NDF residues by treatment (%).

	% DM		% OM	
	3% Fat	6% Fat	3%Fat	6% Fat
Tallow				
Hominy	17.90	19 06	17 71	18 90
Forage	17.91	17 13	17 90	16 81
Fish Oil				
Hominy	19 20	18.35	19 12	18.24
Forage	17 31	20 83	17 06	20 47
WCS				
Hominy	20 45	18 91	19 94	18 05
Forage	17 60	18 31	16 93	17 19

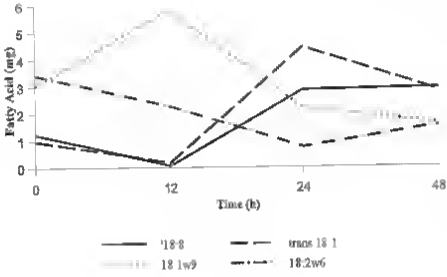


Figure 3-1 Experiment 2. change in 18-carbon fatty acids of corn oil incubated with mixed rumen bacteria *in vitro*

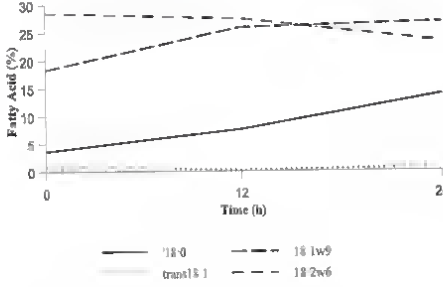


Figure 3-2 Experiment 3 change in 18-carbon fatty acids of six fat sources incubated with rumen bacteria *in vitro*

CHAPTER 4
EFFECTS OF WHOLE COTTONSEED AND A THERAPEUTIC REGIMEN OF BOVINE SOMATOTROPIN ON HEALTH MILK PRODUCTION, AND REPRODUCTION OF EARLY LACTATION DAIRY CATTLE

Introduction

High milk production and low DMI following parturition pose a challenge for both the dairy cow and the producer. Increasing energy density of the diet by adding supplemental fat is common practice on many dairies. In general, milk yield is increased when fat is fed to cows but effects on milk composition and ES of animals has been variable. Dietary fat may enhance reproductive function through increasing cholesterol synthesis (Carroll et al., 1992) and fats high in PUFA may block synthesis of prostaglandins, preventing early embryo loss (Danet-Desnoyer et al., 1993)

Oilseeds such as WCS are used throughout the U S , particularly in the southeast where they are abundant and inexpensive. However oilseeds are high in PUFA, which can depress fiber digestibility under certain dietary conditions (Smith and Harris, 1992) Incomplete hydrogenation of PUFA in the rumen can lead to increased *trans* fatty acids absorbed from the small intestine, which have been shown to depress milk fat synthesis (Romo et al., 1996, Teter et al ,1995)

Whole cottonseed contains a toxin known as gossypol. Gossypol is a polyphenolic pigment produced by the cotton plant and serves as a defense against insect pests. It has long been known that gossypol is toxic to nonruminants and preruminant calves (Colin-Negrete et al., 1996), but was thought to be detoxified in the functional rumen by binding to proteins in rumen fluid. However, additional evidence has shown that dietary gossypol can cause hematological changes in ruminants which may affect health and performance (Gray et al., 1993). More recently, gossypol has been shown to cause abnormalities in sperm from bulls fed high levels of cottonseed products (Chase et al., 1994). Possible effects of gossypol on reproductive function of female ruminants has not been shown.

Recent FDA approval of exogenous bST has led to its widespread adoption by dairy producers to increase milk production. Use of bST early postpartum can exacerbate NES due to the lag between increased milk yield and increasing DMI (Bauman, 1992), and may, therefore, increase the interval between parturition and return to normal cycling. However, doses below that recommended for increased milk production may enhance reproductive function (Stanisiewski et al., 1992).

The purpose of this research was to study the effects of WCS and a reduced dose of bST on milk production and reproduction in early postpartum dairy cattle.

Materials and Methods

Animals and Treatments

One hundred eighty six multi- and primiparous Holstein cows were assigned randomly at calving to one of four treatment regimens in a 2 x 2 factorial arrangement of treatments. Cows were housed in a single freestall barn and were fed TMR for ad libitum intake 2 x daily at 0900 and 1300 h. Cows were milked 3 x daily at 0500, 1300, and 2100 h. Treatments included basal diet with no supplemental fat (no WCS, T0), basal diet plus 15mg/d bovine somatotropin (bST, T2), basal diet with 15% of concentrate dry matter replaced with whole cottonseed (WCS, T1), and 15 % WCS plus 15mg/bST (T3). Animals continued on treatment assignments until diagnosed pregnant, or until 120 d postpartum. Feed samples were collected weekly and analyzed for crude protein, NEL, NDF, ADF, ether extract, mineral composition, gossypol, and fatty acid composition. Diet ingredients and chemical composition are in Table 4-1, and fatty acid composition of diets are in Table 4-2.

Bovine somatotropin (Posilac, Protiva Co., St. Louis, MO) was injected subcutaneously into the tail head area twice monthly (20ml mg/14d). Blood was collected from the coccygeal vein or artery into vacuum tubes containing EDTA as an anticoagulant and preservative (Becton Dickinson, East Rutherford, NJ) three times weekly from 7 (±3) to 83 d postpartum. Cows were weighed bi-monthly through 83 d postpartum and body condition scored (BCS) monthly beginning at d0 (calving), 30, 60, and 90 postpartum based on a five point scale (1=extremely thin to 5=obese).

Time line for reproductive management protocol is presented in Figure 4-1. All cows were injected at 30 d postpartum with $PGF_{2\alpha}$ (25 mg IM; Lutalyse, Pharmacia Upjohn Co., MI) to regress any existing CL and stimulate ovarian activity, and were subjected to timed artificial insemination (TAI) beginning at approximately 65 d postpartum. Cows were assigned to insemination groups by week of calving. Timed artificial insemination program began with intramuscular (IM) injection of 100 µg gonadotropin releasing hormone (GnRH; Cystorelin, Sanofi Inc., KS) at 0600 h, followed seven days later by $PGF_{2\alpha}$, and an additional injection of GnRH at 1600 h two days after $PGF_{2\alpha}$. Cows were inseminated 16 h following second GnRH injection. Pregnancy was diagnosed 36 d post insemination by ultrasound (Aloka Echo-Camera SSD-500; Aloka Co., Ltd., Japan). Pregnancy was confirmed at 45 d post insemination by rectal palpation. Cows diagnosed open after first service were immediately resynchronized and inseminated. Cows diagnosed open by rectal palpation after second service were bred at observed estrous.

Milk weights were taken daily and averaged over 10 d. Milk samples were taken weekly at the afternoon milking and analyzed for fat and protein concentration, milk urea nitrogen (MUN) and SCC. Additional samples were analyzed for fatty acid composition by gas chromatography.

Analysis of Blood and Milk

Following collection, blood was stored in ice for transport, and centrifuged at 3000 rpm for 30 min to separate plasma. Aliquots of plasma were placed in plastic scintillation vials and frozen at -20°C. Plasma was analyzed for progesterone (P_4) by

density lipoprotein (HDL), total triacylglycerol (TAG), TAG fatty acid composition, glucose and plasma urea nitrogen (PUN). Blood was collected 3 x weekly for F_2 analysis beginning d 4 (± 3), and weekly for HDL, PUN, glucose, and TAG beginning d 7 postpartum.

Milk samples were shipped to Southeast Dairy Laboratory, Inc. (McDonough, GA) and analyzed by near infrared spectroscopy for fat and protein percentage and MUN. Milk samples destined for fatty acid analysis were frozen at -20°C. Milk was warmed to mix fat, and 1 ml pipetted into 14 ml plastic tubes. Milk fat was extracted by the method of Chilliard, et al. (1991). To the milk 400μl 95% ethanol v/v, 100 μl 11 N HCL and 5 ml hexane were added. The tubes were vortexed and layers allowed to separate. The upper organic phase was removed and evaporated at 45°C under N. Lipid was methylated according to the method of Maxwell and Marmer (1983). Approximately 20 mg lipid was added to 15 ml Pyrex leak-proof Teflon lined screw cap tubes and dissolved in 2 ml isooctane. To this was added 100μl 2N KOH in methanol. The tubes were vortexed and centrifuged. The lower methanol layer was removed and discarded. The samples were washed twice with 0.5 ml aqueous saturated ammonium acetate, vortexed and centrifuged again, and the lower aqueous layer removed and discarded. Sodium sulfate was added, the tubes refrigerated for 30 min and centrifuged. The top layer was then transferred to vials for GC analysis. Gas chromatography was performed on a Hewlett Packard 5890 GC using an SP2340 (Supelco) 30m x 0.25 mm i.d. capillary column. Injector temperature was set at 175°C and detector at 225°C. Initial oven temperature was 80°C and increase 4 degrees/min to 240°C and held for 20 min.

Plasma used for fatty acid analysis was lyophilized and total lipids extracted according to the method of Folch et al (1957) The chloroform layer of each extracted sample was loaded on Sep-Pak silica gel cartridges which had been conditioned by washing with 10 ml 90% methanol, 10 ml methanol, and 15 ml chloroform Cartridges were washed with 20 ml chloroform under slight vacuum to elute TAG by the method outlined by Elias et al (1991) Chloroform was evaporated in a 45°C water bath under N_2, and TAG methylated according to the method of Maxwell and Marmer (1983) Gas chromatography was performed as describe above for milk fatty acids.

Blood plasma was analyzed for HDL by Sigma Procedure No 352 (Sigma-Aldrich, Inc., St. Luis MO)with the following modifications Plasma was thawed and 250μl pipetted into 13x100 mm borosilicate tubes All samples were prepared and analyzed in duplicate. To this was added 50 μl Sigma Reagent 352-4 (phosphotungstic acid, 30 3 mmol/L, MgCl 100 mm/L) Tubes were vortexed and centrifuged at 3000 rpm for 5 min. Cuvettes were prepared for spectrophotometry with 1 ml Cholesterol Reagent enzyme (Sigma Catalog No 352-50) and allowed to come to room temperature After plasma had centrifuged, 50 μl supernatant was added to cuvettes Cuvettes were inverted to mix plasma and enzyme, incubated at 37°C for5 min, and absorbance read at 500 nm by Perkin-Elmer spectrophotometer Plasma HDL was calculated first based on absorbance of Sigma Cholesterol Calibrator (50 mg/dl) by the following formula

$$A_{test}/A_{blank}/A_{calibrator}-A_{blank}*50*1.2$$

where A=absorbance

test=50μl sample in enzyme

calibrator=50μl calibrator in enzyme

blank=50 μl water in enzyme

and the coefficients represent concentration of cholesterol in calibrator and correction for dilution of samples

Due to variability between runs in the absorbance of the calibrator, a standard curve was prepared using Sigma Lin-trol bovine cholesterol standard (200 mg/dl) The curve was prepared by adding cholesterol standard to an aqueous diluent containing 0.1% sodium azide in the following ratios 0 1, 0 05 0 95, 0 10 0 90, 0.20 0 80, 0 30 0 70, 0 40 0 60, 0 50 0 50, 0 60 0 40, 0 70 0 30, 0.80 0.20, and 1 0 The standards were then prepared and analyzed as described above for samples. Regression coefficients were calculated using Quattro Pro 7 0 with r^2=0.995 for the standard curve The formula for calculating HDL based on the standard curve was y=b-a/x, where y=quantity HDL in mg/dl, b=absorbance at 500nm, a=regression constant, and x=x coefficient of the regression.

Total plasma TAG were analyzed according to the method of Foster and Dunn (1973) Standards were prepared using a stock solution of 500 mg triolein (Sigma-Aldrich Corp , St. Luis, MO) dissolved in 50 ml isopropanol for a final concentration of 1000 mg/ml TAG Standard curve was prepared with isopropanol in 13X100 borosilicate tubes using the following ratios 0 4, 0 01 3 99, 0 025 3 975, 0 05 3 95, 0 075 3 925, 0 1 3 9 Water (100μl) was added to all tubes. Tubes were vortexed and 200μl removed and added to clean tubes

Plasma samples were thawed and 200µl were pipetted into additional tubes All samples were prepared and analyzed in duplicate. Isopropanol (4 ml) was added to standards and unknowns and tubes were vortexed. Adsorptive alumina (400 mg) was then added and tubes were placed on a mechanical shaker for 15 min. Tubes were then centrifuged for 5 min at 3500 rpm and 4°C and 2 ml supernatant was transferred to clean tubes. Saponification reagent (600µl : 10 1.2 0 g KOH water isopropanol) was added, tubes were vortexed, covered and incubated in a hot water bath for 15 min at 65°C Samples were then cooled and 1 ml sodium metaperiodate reagent (3 g 5·0.325 3 5 0 30 anhydrous ammonium acetate,sodium metaperiodate:water·glacial acetic acid w/v) and 500µl acetyl acetone reagent (1 5·40 160 acetylacetone :isopropanol·water v/v) were added Samples were vortexed, covered and incubated again at 65°C for 30 min After cooling 100µl sample were pipetted into microassay plates and analyzed by spectrophotometry at 405 nm Regression of the standard curve was performed by Quattro Pro 7 0 with an r^2 =0 996 and coefficients used to calculate TAG in mg/dl by the formula above for HDL regression.

Plasma urea N and glucose were run simultaneously on a Technicon Autoanalyzer (Technicon Instruments Corporation, Chauncey, N Y) Glucose was analyzed according to the method of Gochman and Schmitz (1972) and PUN by the method of Marsh et al. (1965)

In addition, plasma samples were taken on d 75, 96 and 120 postpartum for analysis of hematocrit, hemoglobin, erythrocyte osmotic fragility (EOF), vitamin E, retinol, retinol phosphate, β-carotene, total gossypol concentration, and concentration of

gossypol isomers. A subgroup of cows (n=60) was sampled every 14 d from d -7 through d 120 postpartum to study changes in hematological profiles over time. Samples were taken in Na heparin tubes every 14 d for gossypol analysis (Becton Dickinson, East Rutherford, NJ) Total plasma gossypol and gossypol isomers were analyzed by HPLC according to the method outlined by Kim and Calhoun (1995) Body condition was scored at d 0, 30, 60, and 90 postpartum Plasma for P_4 analysis was assayed by radioimmunoassay (Knickerbocker et al ,1986) Intra- and interassay coefficients of variation were 6.6 and 6.7%, respectively

Statistical Analyses

Milk yield and composition. Milk yield, fat percentage, protein percentage, MUN and SCC were analyzed according to the Harvey Least Squares and Maximum Likelihood program (1977) The model was

$$Y=\mu+T+C(T)+P+DIM+T*DIM+e$$

where Y=milk yield (kg), fat (%) protein (%) MUN (mg/dl), or SCC (cells/ml)

μ = overall population mean

T = treatment

P = primiparous or multiparous

DIM = days in milk analyzed as a continuous independent variable in a third order regression.

C(T) = cow nested in treatment

e = residual error

The error term for treatment effects was C(T). The term DIM was analyzed using T*DIM as the error, and T*DIM using e as the error term for heterogeneity of regression analysis. The interaction of treatment*parity was not significant, and was dropped from the final model

HDL. The full statistical model may be expressed as

$$Y=\mu+T+L+T*L+C(T*L)+B+T*B+T*B*L+C(T*L)*B+W+T*W+L*W$$
$$+T*L*W+C(T*L)*W+B*W+T*B*W+L*B*W+T*B*L*W+C(T*L)*B*W+e$$

where L=lactation number

W=week of lactation

C(T*L)=cow within T*L

B=batch or run

Due to the complexity of this model and the limitations of the general linear models procedure of SAS, fixed effects were analyzed by SAS proc glm (1987), and the mixed error terms C(T*L)*B, C(T*L)*W and C(T*L)*B*W were generated by the Harvey Least Squares Maximum Likelihood program by absorbing the terms for cow, batch and week, and were used to test the significance of the two- and three-way interactions by subtracting the error sum of squares for the fixed interaction effects from the error sum of squares for the absorbed mixed error terms generated by the Harvey program, and using the error mean square of the mixed effects terms to derive F values of the fixed effects interaction terms

Milk and plasma fatty acids Milk and plasma TAG fatty acids were analyzed by the general linear models procedure of SAS (1987) The general model may be expressed as:

$Y=\mu+T+C(T)+W+T*W+e$, where Y= individual fatty acid

PUN and glucose. The statistical models for these analyses are similar The final model for glucose may be expressed.

$Y=\mu+T+L+T*L+C(T*L)+D+W+T*W+L*W+T*L*W+C(T*L)*W+e$

where D=day of sample run

The final model for PUN may be expressed as:

$$Y=\mu+T+L+T*L+C(T*L)+W+T*W+L*W+T*L*W+C(T*L)*W$$
$$+D+D*W+C(T*L)*W*D+e$$

Non-significant interaction terms were dropped from the final models for these analyses. The mixed error term C(T*L)*W was generated as described for HDL.

Orthogonal contrasts for HDL-cholesterol, TAG glucose and PUN were calculated by hand according to the method described by Snedecor (1951)

Pregnancy Results of pregnancy testing were analyzed by the general linear models procedure of SAS and least squares means for pregnancy by treatment generated. Pregnancies resulting from first, second and the sum of first and second inseminations were analyzed The trial included animals calving from late September through January As the months of September and October were warm enough to induce heat stress, season of calving (warm vs. cool) was included in the model The model may be expressed as

$Y=\mu+T+P+T*P+S+T*S+P*S+T*P*S+B+e$

where P=parity (first or greater)

S=season (warm or cool)

and B=bull

Since the same technician performed all services, technician was not included in the model. Orthogonal contrasts included WCS v no WCS, bST v no bST, WCS x bST, parity, season, WCS x parity, bST x parity, WCS x season, bST x season, WCS x parity x season, and bST x parity x season.

Hematocrit, hemoglobin, erythrocyte osmotic fragility (EOF), α tocopherol, β-carotene, retinol, retinol phosphate, total gossypol, and isomers of gossypol were analyzed by the model.

$$y=\mu+T+C(T)+D+T*D+e$$

where D=day postpartum and was considered to be a class variable. Treatment means and orthogonal contrasts for treatment considered C(T) as the error term.

Accumulated P_4 curves were generated and analyzed by heterogeneity of regression according to the method of Wilcox et al., 1991. Progesterone data were also used to determine the day cows began cycling postpartum, number of cycles per cow, and peak P_4 during luteal phase.

To determine possible influence of gossypol on pregnancy, pregnancy rates to first, second and cumulative first and second inseminations were reanalyzed using plasma gossypol concentrations for d 75, 96, 120 as separate data sets, and mean gossypol levels as continuous independent variables. Model for this analysis included treatment, gossypol, treatment*gossypol, gossypol^2, and treatment*gossypol^2.

Results

Individual feed intakes were not measured in this study. Group averages for DMI were 22.73 for WCS and 22.05 kg/d for no WCS. Milk yield was least for cows consuming WCS, although differences were not significant. However, bST treatment supported milk yield compared to no bST treatment (P=0.0001). Whole cottonseed treatment did not affect milk fat or protein percentage, MUN or SCC. Least squares means for milk yield, fat and protein percentages, MUN and SCC are presented in Table 4-4.

Body condition scores were not different among treatments at calving (Table 4-3). However, BCS were higher among cows receiving the WCS + bST treatment at d 30 postpartum (3.15 vs. 3.10, 3.04, and 2.92 for control, WCS, and bST, P=0.0445). This trend persisted throughout the remainder of the trial.

Treatment least squares means for HDL are in Table 4-4. Figure 4-2 depicts the differences between treatments by day postpartum. Mean HDL concentrations were 77.40, 105.65, 89.63 and 109.15 mg/dl for control, WCS, bST and WCS + bST, respectively. High density lipoprotein cholesterol increased through the first 84 d for all animals. Both bST and WCS (Figure 4-2) increased HDL-cholesterol and the rate of HDL-cholesterol rise (P<0.001). The interaction of bST and WCS also increased mean total HDL-cholesterol compared to control (P<0.001), but the interaction curves were not significant.

Least squares means for PUN, glucose, and TAG are in Tables 4-4. Overall treatment means for PUN are 13.67, 16.35, 13.14, and 14.72 mg/dl for control, WCS, bST and WCS + bST, respectively. Whole cottonseed increased PUN compared to no WCS diets ($P<0.001$), while bST decreased PUN ($P<0.001$). Bovine somatotropin tended to moderate PUN when administered to cows consuming WCS ($P<0.10$).

Overall means for plasma glucose were 60.52, 66.01, 60.15 and 63.14 mg/dl for control, WCS, bST, WCS + bST, respectively. Total TAG means were 15.41, 15.52, 14.25, and 15.85 for control, WCS, bST and WCS + bST, respectively. Plasma glucose was increased by WCS ($P<0.025$), and WCS also tended to increase plasma TAG ($P<0.10$), but bST and the interaction of bST and WCS were not significant for glucose or TAG.

Pregnancy results. Mean pregnancy to two TAI services was 49.46% with a mean embryo loss of 9.38%. Least squares means for pregnancy rate to two TAI services by treatment are in Table 4-5. Main effect of treatments was not significant. However, contrasts revealed a significant interaction of bST x parity x season ($P=0.0216$). Primiparous animals calving during the warm months of the trial were benefited by bST treatment, while bST had no effect on animals calving in the cooler season. No differences were detected among multiparous cows due to treatment. Effects of bST treatment are depicted in Figure 4-3 for cumulative pregnancy rate in primiparous cows. Whole cottonseed had no effect on pregnancy rate.

Milk and plasma fatty acids. Least squares means for milk and plasma fatty acid profiles are in Tables 4-7 and 4-8. In general, cows receiving WCS produced less short

and medium chain fatty acids and higher 18 0 and 18 1ω9 fatty acids in milk compared to cows on the no WCS diet. This pattern of change in milk fatty acids has been noted in previous studies (Storry and Palmquist, 1988 Lubis et al., 1990, Chouinard et al , 1997). Lauric (12:0), myristic (14·0), and palmitic acids (16 0) were all reduced in milk from cows receiving WCS, and increased in milk from cows receiving bST These three fatty acids have been associated with increased risk of coronary heart disease (Enig, 1996) Odd chain fatty acids (15 0, 15 1ω6 17 0 17 1ω8) were all decreased by WCS treatment. These fatty acids are predominantly of microbial origin. It cannot be determined from this evidence if microbial growth was affected by WCS, or if *de novo* synthesis of fatty acids by rumen micro biota was reduced in favor of increased fatty acid uptake from the rumen environment.

Plasma TAG fatty acid composition was less affected by treatment compared to milk fatty acids Most notable was the relative absence of *trans* 18 carbon fatty acids in extracts of plasma TAG compared to milk fatty acid profiles. Palmquist (personal communication) had noted that plasma TAG were 85% of dietary origin Increased 18 0, 18 1ω9, and *trans* 18 carbon fatty acids in the milk but not in plasma TAG on WCS diets may indicate that these fatty acids are found in greater concentration in the NEFA fraction of plasma.

Linoleic acid in milk or plasma TAG was unaffected by treatment. Plasma TAG linolenic acid (18.3ω3) was reduced in cows receiving WCS compared to the no WCS diet (0 56 vs. 0 72 %, P=0 0058). Arachidonic acid (20 4ω6) tended to be higher in plasma TAG from cows receiving bST compared to the no bST treatment group (2 31 vs.

2 00 %, P=0 0645) Mean 18 3ω3 in the WCS diet was 2 50%, whereas the no WCS diet contained approximately 5 27 % 18 3ω3 Linolenic acid is a potential inhibitor of $PGF_{2\alpha}$ synthesis, while 20 4ω6 is the precursor of this prostaglandin (Staples et al , 1998)

Cows consuming WCS showed a more rapid increase in accumulated P_4 compared to other treatments (Figure 4-4) Subsequent analysis revealed that prior to 30 d $PGF_{2\alpha}$ injection, cows on WCS diets had an earlier occurrence of P_4 rise, indicating an earlier return to estrous cycling compared to cows not receiving WCS (Table 4-6) However, this benefit of WCS was mimicked in cows receiving bST (P=0 0538 for interaction). After d 30 $PGF_{2\alpha}$ treatment, cows on WCS diets tended to return to cycling 4 5 d earlier (P=0 0587) and had higher peak P_4 during their luteal phase than other cows (P=0 0272)

Erythrocyte osmotic fragility and total plasma gossypol were greatest on WCS diets (Table 4-9) However, cows receiving WCS + bST had EOF values similar to no WCS treatments Least squares means for control, WCS, bST and WCS + bST treatments were 2 55, 11 13, 1 08, and 2 69, respectively for EOF (P=0 0166 for the interaction WCS x bST) Beginning shortly after calving and continuing through the rest of the treatment period, total plasma gossypol increased in cows receiving WCS alone (Figure 4-5) However, gossypol concentration of cows receiving WCS + bST peaked earlier and declined sooner compared to cows receiving WCS alone (P<0 001)

Because plasma gossypol levels and WCS treatment were highly confounded, least squares analysis which included gossypol as a continuous independent variable did not generate meaningful results. However, heterogeneity of regression of pregnancy rate on gossypol, pooling results by bST treatment revealed a quadratic response to d120

gossypol levels for first service and cumulative pregnancy rate when pooled across bST or no bST treatments ($P<0.10$; Figure 4-6). Pregnancy rate appears to increase when gossypol levels were at or below 2.6 ng/ml, but decreased for higher gossypol levels.

Discussion

No significant effects of WCS were noted on milk yield or composition in this trial. However, bST increased milk yield compared to no bST groups. Increased milk yield is commonly noted when fat is fed (Palmquist and Jenkins, 1980), but is frequently less than might be predicted from increased energy intake (Jenkins, 1993a). Bovine somatotropin generally causes a dose dependent response in milk production.

Dietary fat increased plasma HDL-cholesterol, glucose, and F_4 compared to no WCS diets. Supplemental fat has been noted to increase both cholesterol and F_4 in other studies (Carroll et al., 1990; Caroll et al., 1992). Exogenous bST also increased plasma HDL-cholesterol, but curves of accumulated F_4 were not different between bST and no bST treatments. Somatotropin has a number of effects on the liver, which may include increased lipoprotein and cholesterol synthesis.

Percentage of 18:2ω6 in plasma TAG was unaffected by treatment. However, total plasma TAG was higher in cows receiving WCS, so that total quantity of 18:2ω6 in plasma would be increased. Calculated mg of 18:2ω6 in plasma were 4.81, 4.94, 4.41 and 4.97 for control, WCS, bST, and WCS+bST treatments, respectively. The difference in these quantities, however, may have been too small to effect $PGF_{2\alpha}$ synthesis by the

endometrium. Linoleic acid has inhibited $PGF_{2\alpha}$ synthesis in vitro (Danet Denoyers et al., 1993) and in vivo (Oldick et al 1997). Inhibition of $PGF_{2\alpha}$ synthesis is a double-edged sword. Prevention of premature regression of the CL would enhance the probability of embryo survival. On the other hand, prolonging the survival of the CL in a non-pregnant cow may disrupt normal cycling and prolong the period that animals are open. This latter difficulty may be overcome by therapeutic use of $PGF_{2\alpha}$ and TAI.

Early return to estrous, number of estrus cycles prior to breeding, and high P_4 values during the luteal phase have been indicated to improve pregnancy rates in dairy cattle (Staples et al., 1998). Whole cottonseed treatment did result in higher plasma HDL-cholestrol, accumulated P_4, and an earlier return to estrous, but did not result in greater first, second, or cumulative pregnancy rates in this trial.

In contrast, low dose bST resulted in a 40% increase in pregnancy rates of primiparous animals that calved during the warmer months of the trial. It is possible that low doses of bST may stimulate ovarian activity in young animals still recovering from heat stress. Carryover effects of heat stress have been detected up to two months following exposure (Hansen, personal communications). Primiparous animals under heat stress conditions or recovering from heat stress may represent a target group for therapeutic bST treatment.

The effects of bST on plasma gossypol accumulation of cows fed WCS (Figure 4-5) has not previously been observed. Exogenous bST is known to have a number of effects on the liver (Bauman, 1992), not all of which have been elucidated. It is possible that bST stimulates clearance of gossypol from blood by hepatic tissue. This potential

protective effect of bST may extend to other unexpected benefits to animal health. Further studies are required to more closely examine the effects of bST on liver clearance of gossypol and possible implications for animal health and reproduction.

Although this experiment was not specifically designed to detect potential negative effects of gossypol on pregnancy rates, close analysis of the data by heterogeneity of regression indicates a potential negative effect of gossypol at plasma levels greater than 2.5 ng/ml. The increase in pregnancy rate at lower gossypol levels may be due to the confounding of gossypol with WCS treatment. At low levels of gossypol the beneficial elements of WCS, such as fat and protein, may positively influence pregnancy, but at higher levels of plasma gossypol this beneficial effect is overwhelmed. Further studies are required to determine if, indeed, there exists a dose effect of gossypol on fertility of ruminant females *in vivo*.

Table 4-1 Ingredient and chemical composition of diets.

Ingredient	WCS Diet	No WCS diet
	% of DM	
Corn silage	21 6	22.4
Alfalfa hay	9.1	11 7
Cottonseed hulls	5 6	5 6
Citrus pulp	10 0	10 0
Hominy	15 4	20 3
Distillers grains	9.2	13 4
Soybean meal	8 8	11 4
Whole cottenseed	15 0	---
Vitamin/Mineral mix[1]	5.3	5 3
Chemical composition		
Crude protein, % of DM	17.5	17.9
Undegradeable intake protein, % of CP	35 4	36.7
NEL, Mcal/kg	1 71	1 67
NDF, % of DM	38 1	34 6
Effective NDF, % of DM	25 8	22 0
ADF, % of DM	24.1	21.0
Ether extract, % of DM	6 9	4 6
Ca, % of DM	1 09	1 19
P, % of DM	0 54	0 58
Mg, % of DM	0.35	0 33
K, % of DM	1 51	1 51
Na, % of DM	0.51	0.51

[1] Contained 12 0% Ca, 2 8% P, 2 9% Mg, 1 0% S, 1.6% K, 7 8% Na, 800 ppm Zn, 320 ppm Cu, 800 ppm Fe, 800 ppm Mn, 242000 IU vitamin A/kg, 35,200 IU vitamin D/kg, and 880 IU vitamin E/kg.

Table 4-2 Fatty acid composition of diets (% of total fatty acids[a])

	Control	WCS
14:0	0.26	0.54
16:0	16.28	20.56
18:0	4.55	2.92
18:1ω9	20.57	18.18
18:2ω6	50.34	53.75
18:3ω3	5.27	2.50

[a] results of GC analysis of TMR

Table 4 3. Least squares means of body condition scores of cows by treatment.

	Treatment					Orthogonal Contrasts (P^{bs})		
	T0	T1	T2	T3	SEM	WCS v None	bST v None	WCS x bST
d0	3 08	3 10	3 06	3 23	0 1	ns	ns	ns
d30	3 10	3 04	2.92	3 15	0 1	ns	ns	0 0445
d60	3 14	3 08	2 91	3 13	0 1	ns	ns	0 0741
d90	3 18	3 18	2 90	3.20	0 1	0 0600	ns	0 0580

Table4-4 Least squares means for milk yield, milk composition and plasma constituents

Week	Treatment				Contrasts (P^{bs})		
	T0	T1	T2	T3	WCS v None	bST v None	WCS x bST
Milk Yield and Composition							
MY(kg/d)	32 31	31 58	32.81	32.18	0.3069	0 0001	0 5122
Fat (%)	3.35	3 46	3 43	3 55	0 1956	0 3696	0 9572
Protein (%)	2.90	2 92	2 92	2.92	0 8300	0 9168	0 8005
SCC (x1000)	324	367	403	440	0 7435	0 5340	0 9792
MUN(mg/dl)	15.22	15 22	15 78	14.90	0 3782	0 8042	0 3783
Plasma Constituents (mg/dl)							
HDL	77 40	105 65	89 63	109 15	0 001	0 001	
PUN	13 67	16.35	13 14	14 72	0 001	0 001	0 10
Glucose	60.52	66 01	60 16	63 14	0 025	ns	ns
TAG	15 41	15.52	14.25	15 85	0 10	ns	ns

Table 4-5 Least squares means of pregnancy rates to two TAI services

Treatment	Total Cows	1st TAI	2nd TAI	1st & 2nd TAI
T0	50	37 1	23 6	51 3
T1	45	33 6	26 4	51 8
T2	43	27 1	35 8	51 1
T3	48	27 3	26.2	46.8

Table 4-6. Least squares means ± SEM of reproductive responses to treatment.

	Treatment				Contrasts (*P*=)		
	T0	T1	T2	T3	WCS v None	bST v None	WCS x bST
Days[1] to first P_4 rise (pre $PGF_{2\alpha}$[2])	22.6 ± 1.7	18.5 ± 1.6	18.1 ± 1.4	20.1 ± 1.3	0.4825	0.3505	0.0538
Peak P_4[3] (pre $PGF_{2\alpha}$)	7.8 ± 1.16	8.9 ± 1.06	7.1 ± 0.98	6.7 ± 0.86	0.7138	0.1714	0.4526
Percent cows cycling[4](post $PGF_{2\alpha}$)	27.8 ± 0.1	35.3 ± 0.1	35.0 ± 0.1	42.9 ± 0.1	0.4965	0.5131	0.9880
Days to 1st P_4 peak (post $PGF_{2\alpha}$)	43.0 ± 2.6	37.3 ± 2.4	44.1 ± 2.1	41.1 ± 2.0	0.0587	0.2875	0.5656
First P_4 peak (post $PGF_{2\alpha}$)	10.7 ± 1.2	11.1 ± 1.1	7.8 ± 1.0	11.7 ± 0.9	0.0507	0.2850	0.1030
Second P_4 peak (post $PGF_{2\alpha}$)	8.7 ± 2.2	11.6 ± 1.2	8.9 ± 1.9	8.5 ± 1.9	0.4956	0.4410	0.3642
Percent 2nd cycles[5] (post $PGF_{2\alpha}$)	61.1 ± 0.1	76.5 ± 0.1	76.2 ± 0.1	85.7 ± 0.1	0.2139	0.2243	0.7696

[1] Days to first postpartum rise in P_4
[2] Therapeutic $PGF_{2\alpha}$ administered d30 (±3) postpartum.
[3] Peak P_4 in plasma (ng/ml)
[4] Percentage of cows determined to be cycling (plasma P_4 concentrations >1 ng/ml for at least three consecutive samples).
[5] Percentage of cows having two or more cycles.

Table 4-7 Least squares means of milk fatty acids ± SEM (% of total fatty acids)

					Orthogonal Contrasts ($P=$)		
	Control	WCS	bST	WCS+bST	WCS v. None	bST v. None	WCS x bST
8:0	0.95±0.09	0.66±0.05	0.96±0.04	0.77±0.04	0.0001	ns	ns
10:0	2.52±0.21	1.54±0.13	2.44±0.10	1.82±0.12	0.0001	ns	ns
12:0	3.11±0.23	1.89±0.14	2.96±0.11	2.19±0.12	0.0001	ns	ns
14:0	9.88±0.66	8.31±0.40	9.89±0.31	7.95±0.35	0.0003	ns	ns
15:0	1.12±0.08	0.75±0.05	1.06±0.04	0.72±0.04	0.0001	ns	ns
15:1ω6	0.21±0.02	0.21±0.01	0.24±0.01	0.21±0.01	0.0123	ns	ns
16:0	30.64±1.03	28.16±0.62	30.08±0.48	27.19±0.54	0.0003	ns	ns
16:1ω7	1.07±0.11	0.96±0.07	1.20±0.05	0.91±0.06	0.0104	ns	ns
17:0	0.74±0.03	0.67±0.02	0.70±0.01	0.63±0.02	0.0007	0.0565	
17:1ω8	0.24±0.03	0.24±0.02	0.26±0.01	0.22±0.02	ns	ns	ns
18:0	13.48±1.16	17.78±0.70	13.12±0.54	17.70±0.61	0.0001	ns	ns
18:1ω9	26.98±1.43	30.38±0.85	27.76±0.67	29.88±0.75	0.0063	ns	ns
*trans*18:1	0.31±0.15	0.03±0.01	0.19±0.07	0.38±0.08	ns	ns	0.0215
*trans*18:2	0.02±0.01	0.03±0.01	0.03±0.01	0.04±0.01	ns	ns	ns
18:2ω6	5.79±1.03	5.65±0.61	6.10±0.48	6.67±0.54	ns	ns	ns
18:3ω3	0.51±0.03	0.42±0.02	0.47±0.01	0.42±0.01	0.0021	ns	ns

Table 4-8 Least squares means of plasma TAG fatty acids ± SEM (% of total fatty acids)

					Orthogonal Contrasts (P=)		
	Control	WCS	bST	WCS + bST	WCS v None	bST v None	WCS X bST
14:0	0.39±0.10	0.40±0.08	0.59±0.07	0.64±0.07	ns	0.0083	ns
15:0	0.44±0.03	0.41±0.03	0.48±0.02	0.35±0.02	0.0049	ns	0.0796
16:0	19.44±0.66	20.11±0.57	20.31±0.47	19.95±0.46	ns	ns	ns
17:0	0.44±0.07	0.31±0.06	0.41±0.05	0.30±0.05	0.0355	ns	ns
17:1ω8	0.08±0.02	0.06±0.02	0.05±0.02	0.05±0.02	ns	ns	ns
18:0	23.24±0.60	23.79±0.51	23.75±0.43	23.82±0.42	ns	ns	ns
18:1ω9	16.99±0.71	16.19±0.61	15.52±0.51	15.68±0.50	ns	0.0980	ns
*trans*18:1	0.01±0.03	0.00±0.03	0.01±0.02	0.09±0.02	ns	0.0919	ns
18:2ω6	31.23±1.11	31.71±0.95	30.92±0.79	31.37±0.77	ns	ns	ns
18:3ω3	0.77±0.07	0.57±0.06	0.67±0.05	0.55±0.05	0.0058	ns	ns
20:4ω6	2.04±0.20	1.95±0.17	2.43±0.14	2.19±0.14	ns	0.0645	ns

Table 4-9 Least squares means of hematological data for four treatments ± SEM

Treatment	Hematocrit	EOF[1]	Hemoglobin	α-tocopherol[2]	β-carotene	Retinol P	Retinol	Gossypol
T0	28.82±0 98	2 55±1 88	10.31±0 34	5 27± 1.15	1.21± 0 17	0 57± 0 06	0.37± 0 04	
T1	28 13±0 82	11 13±1 57	10.27±0 28	6 84± 0 96	1 03± 0 14	0 51± 0 05	0 34± 0 03	
T2	28 36±0.60	1·08±1 15	10 34±0 20	4 95± 0 70	0 98± 0 10	0 53± 0 03	0 37± 0 02	
T3	29 47±0.52	2 69±1·00	10 58±0 18	6.34± 0 61	1 22± 0 09	0 60± 0 03	0 32± 0 02	

[1] Erythrocyte osmotic fragility, contrasts were a) WCS (P=0 0005), b) bST (P=0 0007) c)WCS x bST (P=0 0166)
[1] contrast for WCS (P=0 0367)

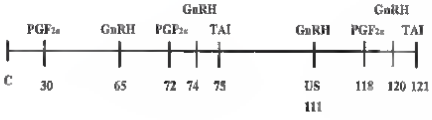


Figure 4-1 Reproductive management protocol

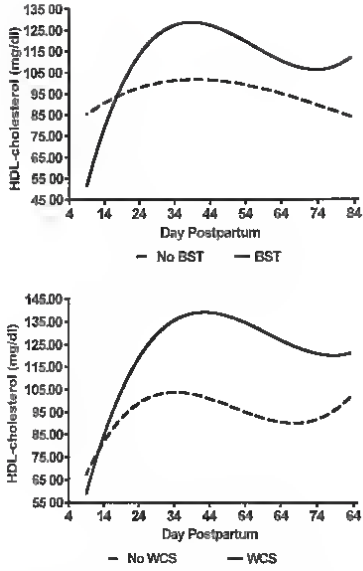


Figure 4-2. Regression curves for plasma HDL-cholesterol (WCS v. none, $P<0.001$, bST v. none, $P<0.001$).

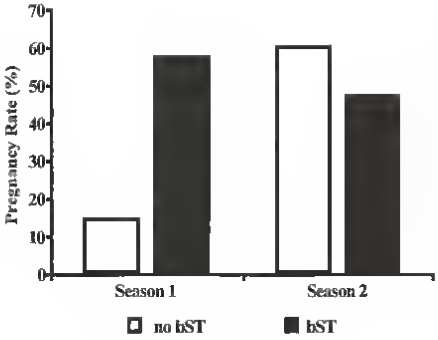


Figure 4-3 Pregnancy rates of primiparous cows (bST x season x parity, P=0.02)

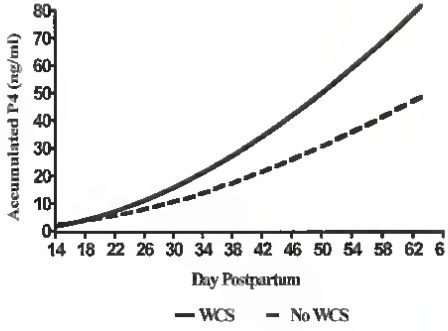


Figure 4-4 Accumulated P_4 curves for cows by diet (WCS v none, $P<0.001$)

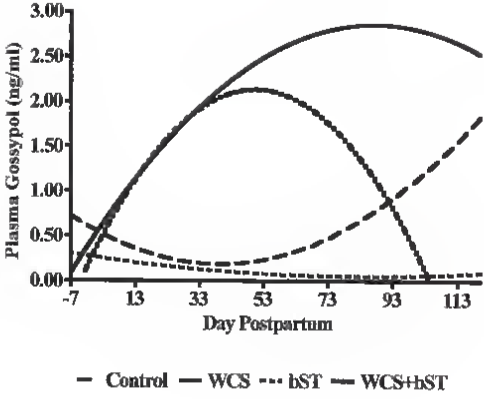


Figure 4-5. Regression of plasma gossypol concentrations by treatment (WCS*bST, $P<0.001$).

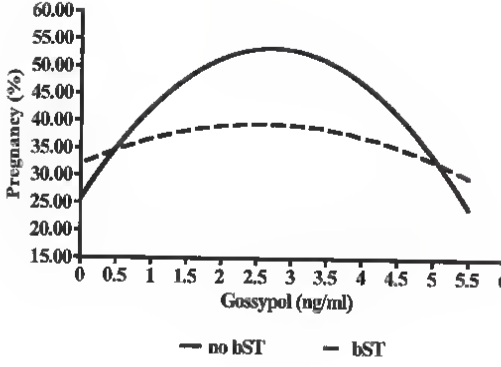


Figure 4-6. Regression of pregnancy rate on plasma gossypol concentration (bST v none, $P<0.10$)

CHAPTER 5
EFFECTS OF WHOLE COTTONSEED AND BST ON OVARIAN FOLLICULAR DYNAMICS IN LACTATING DAIRY CATTLE

Introduction

Increased milk production brought about through the use of bST has led to its widespread adoption by dairy producers. Exogenous bST also stimulates DMI and increases the partitioning of nutrients for milk production (Bauman, 1992). Unfortunately, these increases in production and repartitioning of nutrients contribute to the NES typically experienced by early postpartum dairy cows, which has been associated with reduced reproductive performance (Staples et al., 1991). Evidence of more direct effects of bST on reproductive function have been reported, but considerable variation in responses have been noted. Cows treated with bST have had either increased or decreased plasma P_4 concentrations and altered LH secretion (Schemm et al., 1990, Waterman et al., 1993). Effects of bST may be direct or indirect through IGF-1 stimulation of gonadotropins. Receptors for both bST and IGF-1 have been identified in ovarian tissues. Lactational state has been shown to influence response of ovarian tissue to bST (De La Sota et al., 1993). Dose levels below that recommended for commercial use may improve reproductive performance in lactating cows (Stanisiewski et al., 1992).

Dietary fat is commonly added to dairy rations to increase energy density of the diet to compensate for reduced DMI postpartum and reduce the severity of NES However, evidence has emerged that dietary fat may have other effects to stimulate reproductive response. These effects include increased plasma cholesterol and P_4 (Caroll,et al., 1992), increased number of medium sized follicles (Wehrman et al, 1991), increased size of the preovulatory follicle (Lucy et al , 1993b; Newbold et al , 1997, Rachuonyo et al , 1997), and possible blocking the synthesis of $PGF_{2\alpha}$ which causes regression of the corpus luteum which may result in early embryo loss (Jenkins, 1988)

The objective of this experiment was to study the effects of dietary WCS and low dose bST on ovarian follicular dynamics of lactating dairy cattle during the period just prior to insemination to improve understanding of possible mechanisms of action of these treatments and their effects on reproduction.

Materials and Methods

This experiment was part of a larger study involving 186 dairy cows at the University of Florida Dairy Research Unit in Hague, FL, to study effects of WCS and bST on milk production, animal health, and pregnancy rates during the early postpartum period. Cows were assigned randomly at calving to one of four treatments in a 2x2 factorial arrangement, 0) control or no WCS ration without bST, 1) 15% WCS ration without bST, 2) 208 mg (0 5 ml) bST (Postilac, Protuva Co , St. Louis, MO) injected subcutaneously every 14 d, 3) 15% WCS ration plus bST treatment. The bST treatment

was approximately 50% of the recommended dose for commercial use to increase milk production. All cows were injected at 30 d ±3 postpartum with $PGF_{2\alpha}$ (25 mg im, Lutalyse, Pharmacia Upjohn Co., MI) and estrous synchronized beginning d65 postpartum described previously. Briefly, cows were injected with 100 µg gonadotropin releasing hormone (GnRH, Cystorelin, Sanofi Inc , KS) at 1600 h, followed 7d later by $PGF_{2\alpha}$ again at 1600 h. Another GnRH injection followed 48 h after $PGF_{2\alpha}$ at 1600 h, and cows were inseminated 16 h after second GnRH injection at 0600 h.

A subset of 28 cows (n=7 per treatment) were selected for observation of ovarian follicular dynamics during the TAI period. Ovarian follicular dynamics were monitored daily by ultrasonography beginning the day of first GnRH injection (d 0) and continued through the second GnRH injection (d 9). A real-time Ultrasound unit (Aloka Echo-Camera SSD -500 Aloka Co., Ltd , Japan) equipped with a 7.5 MHZ linear array transrectal transducer was used for examination of ovaries. At each scanning, position and size of follicles (≥2 mm) and corpora lutea (CL) on the ovaries were noted and mapped in relation to each other, and these maps later used to chart changes in structures over the 10-d observation period. Follicles were designated as class 1 (2 to 5 mm), class 2 (6 to 9 mm), or class 3 (> 9 mm). Diameters of the dominant and subordinate follicles and CL also were noted. Cows were scanned also the day after insemination to confirm ovulation of the previously identified ovulatory follicle. Cows which did not ovulate by d 11 were checked again for the succeeding two days, and any animals not ovulating by d 13 were considered anovulatory

Blood samples were collected daily from the coccygeal vein or artery into sterile vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA, Becton Dickinson, East Rutherford, NJ), placed on ice, and centrifuged at 3000 x g for 20 min at 4°C. Plasma was separated and stored at -20°C until P_4 analysis by radioimmuno assay (Knickerbocker et al ,1986). Intra- and interassay coefficients of variation were 6.6 and 6.7%, respectively

Statistical Analysis

Data were analyzed by analysis of variance using the general linear models procedure of SAS (Statistical Analysis System, 1987). Plasma P_4 concentration, total numbers of class 1, 2 and 3 follicles, number and size of CL, and size of dominant and subordinate follicles were dependent variables. Model included treatment, cow nested in treatment, experimental day, treatment*day, and residual error. Main effects of treatment were analyzed using cow within treatment as the error term. Orthogonal contrasts were WCS vs. none, bST vs. none, and the interaction of WCS and bST. Response variables involving repeated measures were analyzed by heterogeneity of regression for day trends by the method described by Wilcox et al., (1990). A single polynomial regression for day was fitted to an individual dependent variable and the difference from fitting individual regressions for WCS vs. none, bST vs. none, and the interaction were tested. Results were declared significant at $p<0.05$

Results

On d 0 of the experiment, 89.3% (25/28) cows had class 3 follicles. In response to the first GnRH injection, 50% (14/28) cows ovulated. More than one CL was detected during the course of the experiment in 14 cows (4, 3, 3, and 4 for treatments 0, 1, 2, and 3, respectively). On day 9, 92.9% (26/28) of the cows had a dominant or preovulatory follicle and 85.7% (24/28) ovulated in response to the second GnRH injection. Of the 4 cows that did not ovulate in response to the second GnRH injection, two underwent CL regression prior to $PGF_{2\alpha}$ administration and ovulated spontaneously. The two remaining cows failed to ovulate even though a dominant follicle was present at the time of administration of the second dose of GnRH.

Two cows were determined to be anestrous based on plasma P_4 analysis performed on samples collected three times weekly from day of calving. Inspite of this, both anestrus cows responded to GnRH administration, developed a dominant follicle, and ovulated following the second GnRH injection. These cows also ovulated a follicle following the first GnRH dose, but failed to develop a functional CL, as plasma P_4 concentrations during the experimental period did not exceed 2.5 ng/ml.

Five of the 28 cows had plasma P_4 values > 1.0 ng/ml on the day of insemination (d 10). Three of the five cows had P_4 concentrations >1.0 ng/ml on d 7, at the time of $PGF_{2\alpha}$ administration, indicating that their CL were not responsive to luteolytic treatment. Following TAI, 17.9% (5/28) cows were diagnosed pregnant by ultrasound on d 36 post insemination.

Neither WCS nor bST affected number of class 1 or class 3 follicles (Figures 5-1 and 5-2). Number of class 2 follicles was significantly different among treatments (Figure 5-3, $P<0.005$) and an interaction of bST and WCS was detected. Number of class 2 follicles was lower and changed little following GnRH administration in cows receiving the no WCS diet. Recruitment of class 2 follicles was enhanced in cows receiving bST and WCS, but the effect of bST on class 2 follicles was absent in animals receiving the no WCS diet. However, this stimulation did not extend to the larger class 3 follicles, from which the dominant follicle is selected.

Growth and size of induced dominant follicles differed among treatments ($p<0.05$) Low dose bST increased the diameter of the dominant follicle following $PGF_{2\alpha}$ injection regardless of diet ($P<0.01$, Figure 5-4) Dominant follicles of cows injected with bST but consuming the no WCS diet exhibited greater dominance, reflected in decreased number of class 2 follicles on the ovaries of cows receiving this treatment. Growth curves for subordinate follicles likewise were not parallel across treatments (Figure 5-5, $P<0.005$). Effects of diet ($P<0.005$) and a WCS*bST interaction ($P<0.01$) were detected for size of the subordinate follicle As with class 2 follicles, the dominant follicle of bST treated cows on the no WCS diet exhibited greater dominance, resulting in a smaller size subordinate follicle (Figure 5-5) Dominant follicles of cows receiving WCS without bST, however, did not suppress the growth of subordinate follicles to the same extent.

Sixteen cows which had a plasma P_4 profile similar to that expected during a normal diestrous phase, indicating that a functional CL was present throughout the experimental TAI period from d 0 to d 7 Twelve cows were excluded from the analysis

due to spontaneous regression of CL one to two days prior to $PGF_{2\alpha}$ injection, or had been in estrus at the time of the first GnRH injection or immediately following. Since 11 of the 16 cows had more than one CL, either naturally or induced, P_4 estimates were adjusted for number of CL using CL number as a covariate for regression analysis. Analyses were performed using data for d 0 to 10 for data associated with the luteal phase (d 0 to 7), and during the period of CL regression (d 7 to 10) to examine effects of before and after induction of CL regression.

Regression curves for plasma P_4 concentration from d 0 to 7 and 0 to 10 were different among treatments, and a WCS*bST interaction was detected ($P<0.01$, Table 5-1). Cows treated with bST and consuming WCS had higher P_4 concentrations in plasma and maintained those levels during the diestrous phase. In contrast, cows on the no WCS diet treated with bST had lower initial P_4 concentrations, but then experienced more rapid increases in P_4 (Figure 5-6). During the period of CL regression, an overall significant effect ($P<0.01$) effect of WCS diet was detected. However, differences in plasma P_4 were not detected among treatments when d 8, 9 and 10 were considered (Table 5-1). Regression of the CL did not differ among treatments and was complete by d 9 in both WCS and no WCS groups.

Cows with multiple CL were evenly distributed among treatment groups. Mean CL number was 1.55 ± 0.08. Diameter of CL also did not differ among treatments, although cows receiving WCS had a tendency ($P=0.06$) to develop slightly larger CL than cows not fed WCS diet (33.9 vs. 31.6 mm).

Discussion

The low pregnancy rate for cows on this experiment (17.9%) within the observed group compared to overall first service pregnancy rate of the main group (n=186; 34.9%) can be explained by the stress induced by daily rectal examination during the insemination period and the day following insemination

Increased number of preovulatory follicles during the second follicular wave among cows treated with the recommended dose of bST (500mg/14d) has been reported previously (Kirby et al , 1997). Earlier return to estrus postpartum was observed in cows receiving lower doses of bST (41.1, 5 mg/d) compared to higher doses (57.1 d, 14 mg/ml) and untreated controls (50.1 d) in the experiment of Stanisiewski et al., (1992).

Number of class 1 and class 3 follicles did not differ due to treatment in the current experiment, although number of class 2 follicles was influenced by bST dose depending on diet. Cows receiving bST and consuming WCS had more class 2 follicles than bST treated cows fed no WCS diets This suggests that bST stimulates a more dominant ovulatory follicle, an effect which was suppressed by WCS. Wehrman et al., (1991) observed that WCS stimulated number of medium size follicles. Somatotropin increased the population of class 2 follicles during the first follicular wave in lactating cows (Lucy et al , 1992) and increased plasma IGF-1 concentration in plasma (Newbold et al., 1997). Increased IGF-1 is the likely mediator of altered follicular dynamics due to bST administration in this experiment.

Size of the induced dominant follicle in bST treated cows during the final stage of growth was significant in the current experiment. A WCS*bST interaction was detected for size of the subordinate follicle, supporting the existence of a differential effect on follicular dominance due to bST depending on diet. In the absence of WCS, bST treated cows had smaller subordinate follicles than cows consuming WCS. This corresponds with the effects observed on class 2 follicles. Similar findings were reported by Lucy et al., (1993b) in cows consuming CaLCFA. The mechanism by which bST and diet interact to modify ovarian follicular dynamics is not known. Alteration of LH secretion patterns by bST in lactating cows has been noted (Schemm et al., 1990, Waterman et al., 1993), and this may alter ovarian function.

The dosage and time of administration of bST relative to calving seem to affect reproductive responses in lactating dairy cows. In the present study, administration of 208mg/14 d bST, or an average of 14 mg/d, began approximately seven days postpartum. Cows administered larger doses (25 mg/d) beginning at d 35 or d 70 postpartum and continuing to 200 d had increased plasma P_4 and LH concentrations (Schemm, et al., 1990). Levels of 40 mg/d beginning at d 32 or 85 postpartum to d 180 decreased plasma P_4 and basal LH concentrations (Waterman et al., 1990), indicating a dose response to bST in reproductive response.

Studies have shown that bST increases plasma IGF-1 in lactating cows (De la Sota et al., 1993; Newbold et al., 1997), and increased the number of follicles 6 to 15 mm in diameter (De la Sota et al., 1993). *In-vitro*, IGF-1 and IGF-2 consistently stimulated P_4 synthesis by granulosa cells of several species, including bovines (Spicer and

Echternkamp, 1995) Progesterone and IGF-1 concentrations in serum are positively correlated (Spicer et al., 1990, 1993). Somatotropin receptors are found in abundance in bovine CL, particularly in the large luteal cell (Lucy et al., 1993b) Thus, bST effects on plasma P_4 and ovarian dynamics may be both direct and indirectly mediated through IGF-1

Supplemental dietary fat increases serum concentrations of naturally synthesized somatotropin, insulin and cholesterol (Thomas et al., 1997). It was also observed that concentrations of cholesterol and IGF-1 in follicular fluid in the same experiment when supplemental fat was fed. Increased hepatic synthesis of cholesterol in response to increased absorption of fat from the small intestine may have provided increased precursor to the CL for P_4 synthesis. Burke et al., (1997) reported increased P_4 concentrations in plasma of cows fed Menhaden fish meal

Lactating cows fed fish meal have attenuated uterine $PGF_{2\alpha}$ secretion when luteolysis is triggered under experimental conditions (Cochlo et al., 1997). Menhaden fish meal contains approximately 8% fat, including significant amounts of very long chain n-3 fatty acids, which may inhibit $PGF_{2\alpha}$ synthesis

Linoleic acid has been shown to inhibit prostaglandin synthesis through competitive inhibition of cyclooxygenase (Danet-Denoyers et al., 1993) Whole cottonseed contains more that 50% 18:2ω6, however, biohydrogenation in the rumen of 18:2ω6 in cottonseed may be as high as 70 to 90%, limiting the availability of this fatty acid for intestinal absorption (Palmquist and Jenkins, 1980) Although no difference was noted in 18.2ω6 concentration between cows receiving WCS and no WCS diets in the

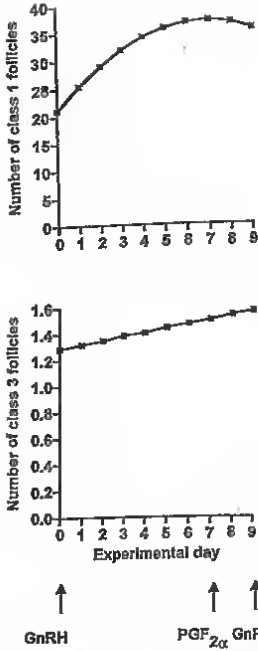
larger experiment, total plasma TAG were increased on the WCS diet, which would have increased the total milligrams of 18:2ω6 absorbed.

Current results indicate possible benefits of bST treatment in combination with dietary fat on certain elements of reproductive function, namely, increasing the number of medium sized follicles from which dominant follicles may be selected.

Table 5-1. Post luteal phase plasma progesterone concentration from d7 through 10 of TAI protocol

Treatment	Progesterone (ng/ml), mean±SE			
	d7*	d8	d9	d10
T0	6 04 ±1 41	1 28 ±0 43	0 71 ±0.45	0 62 ±0 11
T1	11 80 ±1 64	2 35 ±0 54	0 97 ±0 52	0 60 ±0 13
T2	8 23 ±1 46	1 91 ±0 43	1 47 ±0 45	0.50 ±0 11
T3	11 33 ±1.28	2 19 ±0 40	1 19 ±0 40	0 72 ±0 10

*Contrast for WCS v none $P<0.02$



Figures 5-1 and 5-2 Change in number of class 1 and class 3 follicles

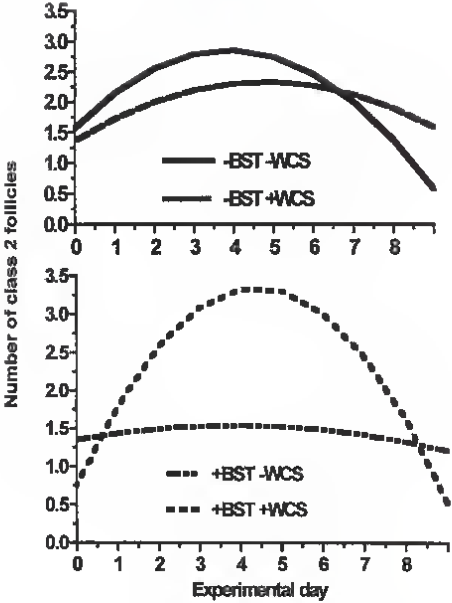


Figure 5-3 Number of class 2 follicles by treatment (WCS*bST, $P<0.005$)

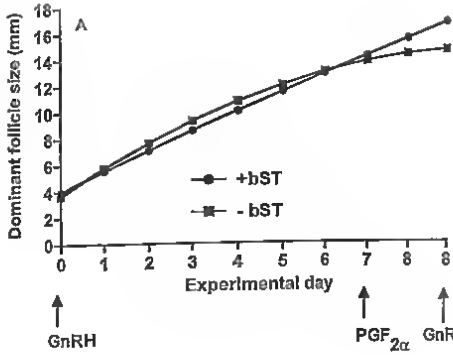


Figure 5-4 Growth and size of induced dominant follicle (bST v none, $P<0.01$).

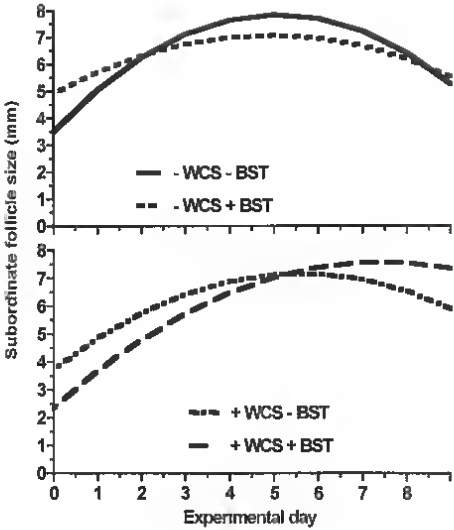


Figure 5-5 Growth and size of subordinate follicle (WCS v none, $P<0.005$, WCS*bST, $P<0.01$)

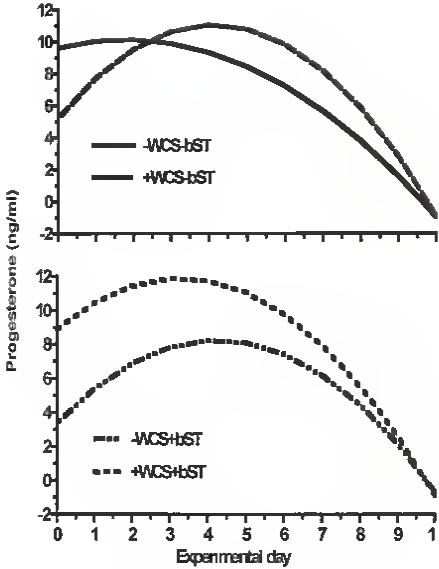


Figure 5-6 Change in plasma P_4 concentrations from d 0 to 10 (WCS v none, $P<0.01$).

CHAPTER 6
NUTRIENT AND MANAGEMENT OPTIONS FOR FLORIDA DAIRIES

Introduction

The dairy industry in the US faces many challenges, not the least of which is maintaining profits in the face of increasing feed prices, environmental regulations, and decreasing federal support for agriculture. The 1996 Federal Agricultural Improvement and Reform Act (H.R. 2854) will phase out most crop subsidies over seven years. Crops affected by this change in policy will include corn and soybeans, which have been staple commodities for most livestock feeds. This legislation will also phase out the Dairy Support Price program over four years.

In addition, environmental regulation of livestock waste disposal has become a major public concern, and much of the focus for this issue in Florida has been on dairies. Dairymen are now required to develop manure disposal systems in order to comply with Florida Department of Environmental Protection water quality standards (Twachtmann, 1990). This has led to considerable research efforts which emphasize nitrogen (N) recycling and address such issues as maximum carrying capacity and nutrient uptake by crops (Van Horn, 1992, Frausse et al., 1996).

Pressures within dairies also affect the financial health of the operation. Because of the large size of these operations and high production levels of the cows, many dairies milk on a three times per day basis, requiring three full time shifts in the parlors and barns.

Most dairies in Florida manage animals under a semi-intensive or intensive system. Animals may have access to some pasture, but, depending on their stage of lactation may spend most of their time confined to freestall barns with their feed and water provided to them. Dairymen have expressed renewed interest in more extensive management systems such as rotational grazing to reduce capital investment costs while complying with environmental regulations (Staples, 1995). Three so called "grazing dairies" are currently being managed in Florida. Also, an ongoing study at the University of Florida's Dairy Research Unit, currently in its third year, examines effects of seasonal grazing of improved pastures on feed intake, nutrient supplementation, and milk production.

Lactating dairy cows require high quality, high energy and protein feeds in order to produce to their potential. Feed costs are thus the most significant expense to the dairy, comprising 50 to 70% of the gross income from milk (Buchanan, personal communications). It has become increasingly apparent in recent years that current nutrient definitions and requirements are inadequate, and require reexamination in order to improve animal health and efficiency of feed conversion to milk (Hall, personal communications).

Also, reproduction has become a major issue of concern to dairymen in Florida. Artificial Insemination (AI) is commonly part of reproductive management. High peak production shortly after calving, combined with extended periods of heat stress

contributes to low conception rates to AI on Florida dairies, and calving intervals are longer than in cooler areas of the country (Badinga et al ,1985) Cows typically return to AI service four to five times, and cows which do not conceive until mid- to late lactation do not produce to their lifetime potential. Reproductive failure is a common reason for involuntary culling and, because the replacement rate on many Florida dairies is 35 to 40% (Webb, 1994), poor conception rates can mean increased expenditure for replacement animals and reduced opportunities for genetic improvement

Considerable research efforts have been devoted to resolving these issues In the area of reproduction, for example, many technologies have been developed, including nutritional supplements (Lucy et al., 1991b Garcia Bojalil 1993 Burke, at al., 1997) and hormonal treatments (Oldiek, et al , 1997), while other approaches are still being studied Although some of these technologies are indeed effective, little attention has been given to their financial impact on the farm.

To examine such questions, a model was developed based on an existing dairy in North Central Florida using linear programming. The model was designed to express current activities, constraints, and costs and was expanded to evaluate additional management scenarios and technologies discussed in previous chapters in order to study their affects on milk production, costs and farm income

Materials and Methods

Data Collection

The model was developed based on an existing dairy in North Central Florida. An interview was conducted with the dairy owner to identify major activities and constraints of the operation. Additional information was obtained from published data.

According to the dairy owner, most cows have access to pasture while lactating. Dry cows were maintained on pasture, while 380 acres were under development to provide rotational pasture for heifers. Fresh cows (high group) were maintained in total confinement, with no access to pasture.

The dairy owner stated that 560 acres of the farm were currently in crop production. The dairy produced mainly corn for silage and a winter annual cereal grain, either rye, oats or wheat, also to be used as forage. The dairyman was planting bermudagrass to produce hay. The main purpose of the crops, however, was for recycling of manure N to comply with environmental regulations. The dairy owner estimated average corn silage harvest was approximately 4.80 MT DM/ha, while cereal grain yields 2.54 MT/ha. Bermudagrass was estimated to produce 5.46 MT/ha of hay per acre (Van Horn, 1992). Nitrogen uptake by crops was estimated to be 65 kg/ha corn, and 58.59 kg/ha cereal or bermudagrass (Sanderson and Jones, 1997), while P uptakes were estimated to be approximately 13.3 kg/ha for the three crops (Van Horn, 1992). All replacement heifers were raised on farm. Cull rate on the dairy was 30%, with a death loss of approximately 5%. Cull cows were sold at $380/head. Veal calves were sold at 1

d of age for $5/head. The estimated cost of raising a replacement heifer to first calving was approximately $1100 and is thought not to differ from purchase/sale price (Bachman, personal communication). For purposes of the model, annual calf crop was assumed to be 90% with 50% of calves born as bulls. Labor was estimated to cost $9 per person hour (Thomas et al., 1996). Milk price was based on Florida market order price for May 1997 (DeLorenzo, 1997).

Model Construction

Figure 6-1 is a schematic representation of the interacting forces which influence management decisions on a dairy. Because dairies in Florida were principally businesses and managed separately from concerns affecting the family, the component typically referred to as "farm household" in livelihood systems analysis has been replaced with "farm management" (Hazel and Norton, 1986). Market has been disaggregated to separate functions of milk processors, credit agencies, government and the local community.

The farm is primarily in the business of producing milk. In order to sustain production levels throughout the year, it is necessary to maintain animals of different ages and in different stages of their production cycle. Animals in the system therefore include adult cows in four stages of lactation: high (to 90 DIM), medium (91 to 210 DIM), low (211 DIM to dry off), and dry cows; cull cows, replacement heifers, and bull calves. Although most Florida dairies use AI as their principal means of breeding, a few bulls were generally maintained on farm. However, as these bulls were typically housed and fed with the cows they were expected to breed, they cannot adequately be separated from that group for analysis.

Farm management allocates labor for each activity on the farm. Cows produce milk, all of which is sold to processors. Cull cows were sold for meat, and bull calves were sold primarily for veal. On dairies which are engaged in genetic improvement programs, bull calves may also be sold for breeding stock or kept and their semen sold. This, however, was not a common practice, and was not included in the model. Animals also produce manure, which was flushed from the milking parlor and free stall barns into a holding lagoon and then sprayed on crops. This activity was primarily to satisfy government water quality regulations which were designed to monitor and control runoff of N. In the Okeechobee region, phosphorous (P) is the monitored manure nutrient. The crops produced on this farm contributed feed in the form of silage and hay to the animals. In addition to cash for milk sold, the market also provides credit in the form of free-floating loans, labor, feed commodities for concentrate, additional hay, seed, and fuel. The government receives taxes from the farmer, and also regulates both the dairyman and the milk processor in terms of milk quality and environmental standards. The surrounding community not only purchases milk from the market produced by dairies, but also was affected by odors produced by the animals. Labor flows from the community through the market to the dairy.

In order to test the value of hormonal treatments to improve fertility, timed artificial insemination (TAI) was introduced as an alternative technology and compared to a Control Option, where reproductive management depended upon breeding cows at observed estrus. Bovine somatotropin (bST) was included as an additional management option for its expected effects on milk production, nutrient requirements, and DMI

Cows were segregated into high, medium and low production groups The average annual production for a cow in each group, assuming that there would be cows in each group throughout the year was calculated based on the estimated peak and rate of decrease in production as each animal progresses through its lactation cycle (NRC, 1989). Dry cows were incorporated into each production level in order to facilitate tracking of animals through each stage of production.

Since feed is the largest single cost to the dairy, diets were formulated for each production level based on feeds available on farm and commonly purchased commodities Nutrient requirements and feed compositions were based on NRC Requirements of Dairy Cattle (1989) and experimental data Estimates of feed costs were based on January 1997 prices (Feedstuffs, 1997) for purchased commodities, and estimated costs of production of on farm forages Pasture requirements for dry cows and heifers were based on estimates from Large Dairy Herd Management (Van Horn,1992) for N and P disposal

Equations

The model was analyzed by the Quattro Pro 6 0 optimization program in Windows 3 1

The objective function of the model was

$$MAX \sum inc_y$$

where inc_y=net annual farm income for year y such that

$$inc_y = \sum_i a_i p_j$$

where a_i=level of activity i

P_j=price j per unit activity i

Activities within the matrix included milk production at two potential RHA with cows disaggregated into high production or fresh cows, medium production or mid-lactation cows, low production or late lactation cows, and dry cows. Additional activities included production of heifer calves and on-farm replacements, sale of heifers, bull cows, and vealers; purchase of replacements, crop production, labor hiring, feeding on-farm crops, sale of on-farm crops; purchase of commodity feeds, and transfer of land from either pasture or cropping use.

Constraints on the system included

$$land_b = \sum H_k G_d$$

where $land_b$= hectares needed for pasture b

H_k= head of cattle in group k

G_d= hectares required for grazing d per cow in group k

$$land_c = \sum R_c Mn_f \leq T_b$$

where $land_c$= hectares in crop production in season c

R_c= tonnes per hectare of crop c

Mn_f= tonnes of mineral f (N or P) uptake per ton crop c

T_b= hectares transferred to or from pasture b

$$Barn_g = \sum Lact_m * 18.46$$

where $barn_g$= m^2 available floor space in free stall barns, assuming

18.46 m^2 floor space per cow

$Lact_m$= lactating cows m

Milking parlor requirements were based on average amount of time in minutes/day, a lactating cow was expected to spend in the parlor, using figures established for the type of parlor on farm, which was a double 35 parallel design (Thomas, et al., 1996). Parlor was further disaggregated into winter and summer parlor use, assuming that more animals would calve and enter the lactating herd during the cooler months of the year

$$parlor_s = \sum Last_m * Min$$

where $parlor_s$=parlor time during season s per lactating cow m

and Min=minutes/day

Nutrient balancing for the farm was accomplished for N and P by season based on the following formulae

$$summerN = \sum H_h (\sum Feed_r (DIP_{feed} + UIP_{feed}) - (N_{milk} * 0.4) * ha_h N_c + store_n = 0$$

$$summerP = \sum H_h (Feed_r P_{feed} - P_{milk}) - ha_h P_c + store_p = 0$$

where summerN = nitrogen flow during warm months

$Feed_r$ = tonnes of feed ingredient r

DIP_{feed} = tonnes of DIP N in feed ingredient r

UIP_{feed} = tonnes of UIP N in feed ingredient r

N_{milk} = tonnes of N in milk

$store_n$ = tonnes of storage N from previous season

$store_p$ = tonnes of storage P from previous season

N_c = tonnes of N in crop c

P_c = tonnes of P in crop c

summerP = phosphorus flow during warm months.

P_{feed} = tonnes of P in feed ingredient n

P_{milk} = tonnes of P in milk

assuming feed N is 16 percent of feed protein, which may be divided into either undegraded intake protein (UIP) and rumen degradeable intake protein (DIP), milk N is 15 5 percent of milk protein, which is assumed to be an average of 3 20%, and that adult cows do not accrete significant amounts of N, therefore the difference between feed N and milk N is excreted N (Van Horn, 1992) Further, it was expected that 60 percent of N is volatilized before application to crops, and that summer growing season (specific to Florida conditions) is 9 months or 67% of the year Summer P excretion was calculated with similar assumptions except that no volatilization was expected Winter nutrient flows were calculated under the same assumptions as for summer N and P, except that the growing season for winter annuals was expected to be three months or 33 % of the year No allowances were made for luxury consumption by plants, or soil holding capacity Storage was added to the model for carryover of nutrients from summer to winter but nutrient balance must be met annually Thus, nutrient balance was forced by setting either N or P equations equal to zero

Nutrient requirements for lactating cows were estimated based on ± 5% deviation from requirements calculated by NRC (1989) for mature cows approximately 600 kg BW, subject to milk production/day and estimated days pregnant. Nutrients included in the ration balancing included DMI, net energy for lactation (NEL), NDF, non-structural carbohydrate (NSC), DIP, UIP, Ca, and P For all nutrient balancing except NEL, the general equation was

$$req_n = \sum H_r nut_v - Feed_r nut_v$$

where req_n = ±5% the requirement for nut, per year

nut_v = tonnes of nutrient v

Requirements for NEL were calculated similarly

$$req_{mcal} = \sum H_r NEL_{mcal} - Feed_r NEL_{mcal}$$

where req_{mcal} = ± 5% the megacalorie requirement

NEL_{mcal} = megacalories net energy for lactation in feed ingredient r

Total adult cows on farm were calculated by the equation

$$cow = high + med + low + dry$$

where high = high production cows

med = medium production cows

low = low cows

dry = dry cows

Cows were moved from one production level to another by the following

$$high = 0\ 32med$$

$$med = 3\ 05low$$

$$low = 1\ 32dry$$

Given four free stall barns, each with a capacity to hold 500 cows, ratios were determined assuming that most of the cows on the farm would be in mid-lactation or the medium production range, and that this would be approximately three times the number of late lactation cows. For every mid-lactation cow there would be 0 32 early lactation cows, and for every dry cow there would exist 1 32 late lactation cows. Dry cows were expected to

comprise 15% of total adult cows. Similarly, heifers were divided into calves and springers, with approximately 0.8 springer for every heifer calf.

Milk production was calculated by the equation

$$milk_y = \sum H_k prod_k$$

where $milk_y$ = annual farm milk production in metric tonnes

$prod_k$ = annual milk production per cow in group k

Labor was calculated

$$labor = \sum a_i L_i$$

where L_i = person hours per activity i

Person hours were estimated based on information provided by the dairyman as to total number of employees and employees/task.

The model was further expanded to examine the potential value of dietary fat and low dose bST to decrease calving interval and replacement rate. Calving rate was adjusted for calving interval using the equation of Esslemont (1992), so that these effects could be estimated on an annual basis. Since data are still being compiled on the effects of dietary fat and low-dose bST, sensitivity analysis was used for these parameters.

Model Validation and Potential Uses

The base model was validated by comparing solution results with data provided by the farmer regarding total number of animals, animals/production group, and stated RHA objective (20,000 lbs/c/yr). The model may be used to examine a number of issues of concern to dairy farmers and agricultural scientists such as policy changes and

introduction of new technologies. Of particular interest were examination of nutritional supplements and hormonal therapies developed to increase cow fertility.

Results

The base model showed virtually no difference in outcomes when milk production or gross margin were maximized (data not shown). Milk is the major source of income for dairies. Further changes to the model were tested with maximizing gross margin or minimizing total costs as the objective function.

In addition, the model was tested under two different scenarios, one which required N recycling as a constraint, and another in which both N and P (N+P) balance was a constraint. Previous runs had included only P as the mineral to be balanced without the option to purchase additional fertilizer N, but results showed that N became limiting for plant growth, and, hence, maximum P uptake would not be achieved. Therefore, the N+P model was designed such that the program had the option to purchase additional N fertilizer in order to balance for both minerals. Results of both N and N+P models are in Table 6-1.

Land area and barn space were limiting under both N and N+P scenarios. Regardless of whether N or N+P was binding, solutions showed that additional land would need to be transferred from existing pasture to crop production. Quantity of land required for crop production varied depending on season of production and whether N or N+P balance was binding and on season of production. Under N constraint, land

transferred from pasture to crop production in summer was 150 58 ha, while in winter, land transferred to crops was 355 25 ha, due to the lower production expected from cereal grains compared to corn silage and bermudagrass. An additional 48.29 ha was needed to produce crops above that required under N only conditions when N+P were binding in summer, but the land required for winter production was similar (355 99 ha).

When minimizing total costs was the objective function, solutions for both N and N+P constraints were similar. Land was again binding under both mineral constraints as the model attempted to minimize feed costs by producing as much forage as possible on farm. Shifts in crop production were noted when cost minimization was required vs. income maximization under both N and N+P. Corn silage and bermudagrass hay production increased at the expense of cereal hay production when cost was minimized under N balance. Silage production increased 3,960 17 MT and bermudagrass production increased 3 710 38 MT, while cereal hay decreased 896 10 MT. These shifts were accomplished by a shift in N utilization.

Replacement management also differed whether income was maximized or cost was minimized. When income was maximized, all heifers were sold and replacements purchased. However, when cost was minimized, the program elected to maintain heifers on farm. Webb (personal communications) had recommended that, barring decisions regarding genetic improvement, it was more economical to sell farm-born heifers and purchase all replacements. The model did not include genetic improvement as a consideration for decisions regarding replacement management, although this is typically the reason given by dairymen who raise heifers on farm. Since the price differential

between raising and purchasing replacements was minimal, the decision to purchase pregnant replacements rather than raise heifers may have been due to the land area required by growing, non-productive heifers vs that for older animals ready to enter the milking herd.

In order for the program to provide other than a null solution when cost was minimized, a minimum annual farm milk production was set at 22,000 MT, based on milk production under N+P balance (22,621 37 MT). Gross margin of the farm enterprise was highest when income was maximized under the N balance constraint ($7,070 698), and lowest when cost minimization was the objective function ($5,007 431). Under the N+P balance model with income maximization net farm income decreased $790,717, due to multiple factors resulting in increased costs per cow and per MT milk With N+P balance constraint operative, the cost minimization solution reduced farm annual gross margin to $4,490,304 Under all scenarios, both TAI and bST were selected as part of the optimum solution, however, TAI usage was substantially reduced when N+P were constraints, and bST usage was reduced under N constraint when cost minimization was the objective function.

Under both scenarios, TAI was selected over Control for reproductive management. Reproductive failure is a leading cause of involuntary culling followed by feet and leg problems, and constitutes 29% of involuntary culls in Florida (Webb, 1994) Based on experiments performed at this and other dairies, TAI reduces cull rate from 42 to 30%, or a 12% decrease in salvage cost (Thatcher et al., 1997) Timed AI has been used on this dairy for two years as part of a UF Dairy and Poultry Sciences research

program. Estimated cost per cow of the hormone series is $10. However, cull and replacement rate did not benefit from use of TAI under the conditions of the current model. Timed AI increased the number of animals calving and entering the milk herd in summer, thus increasing annual farm milk production.

Bovine somatotropin was selected over the no-bST option, because of its high return over cost. The base model included estimated bST effects only on milk production, nutrient requirements and feed intake, and does not reflect potential effects on reproduction, which were still being evaluated. However, even when expected calving rate due to bST use was experimentally reduced to 80%, the model still selected bST over no-bST.

Subsequent models were run using income maximization as the objective function and only N as the mineral for regulation, since these were considered to be the conditions under which the dairyman was operating, eliminating each of the hormonal treatments sequentially so that outputs may be compared. Results are in Table 6-2. In one such model, neither bST nor TAI were available options (-TAI, -bST), in another, bST was available, but not TAI (-TAI+bST), and in another TAI was available, but not bST (+TAI,-bST). These scenarios were then compared with the full model in which both treatments were available as options.

Use of bST increased cost/cow ($1.77 vs. $3.23) but decreased cost/MT milk ($193.76 vs. $179.66), and increased total income compared to models in which bST was not available. Increased efficiency of feed conversion to milk has been attributed to exogenous bST, resulting in reduced feed-over-milk costs (Bauman, 1992). Bovine

somatotropin increased requirement for crop land compared to the no bST option due to the expected increase in feed intake and subsequent increase in fecal N output (330 73 vs 303 98 ha) Highest income was achieved when both treatments were available to the program, with lowest cost/ MT milk ($159 58)

Through the use of linear programming, ration formulation to maximize profits produced formulations which met specified nutritional criteria and constraints. The program was offered a selection of 21 commodities including corn silage and cereal hay, grown on farm, bermudagrass hay, either purchased or grown on farm, alfalfa, cottonseed hulls, hominy barley, citrus pulp, peanut meal, soybean meal, cottonseed meal fish meal, urea, tallow, Megalac®,WCS, deflourinated phosphate, $CaCO_3$, bone meal, and trace mineral salt. A limit was placed on WCS such that the diet could not comprise more than 15% of total diet DM due to concerns over effects of unprotected polyunsaturated fatty acids in the rumen and possible toxic effects of gossypol, an antiquality factor A urea constraint was introduced limiting urea to 1% of ration DM to prevent feeding of toxic levels Also, a minimum roughage constraint was added and treated as a nutrient, to prevent digestive upsets and other metabolic disorders

Dietary fat was used by the program to balance energy requirements of lactating cows. The fat source of choice was WCS due to its relatively low cost compared to other dietary ingredients which were also high in fat and/or protein. Whole cottonseed was fed at the maximum limit allowed (15% of DM) to high production cows As production level declined, forage, especially from on farm sources, began to replace more expensive purchased concentrate ingredients and alfalfa hay As production decreases through the

lactation cycle, nutrient requirements also decrease as demands on the animal to support milk component synthesis declines. The program was designed to optimize feed costs along with other demands on the farm system.

The expanded model did not select either supplemental fat or therapeutic bST as options to increase animal fertility and reduce calving interval and replacement rate by 5%. As additional information is made available, the model may be altered to reflect more accurately the expected benefits of these interventions on farm income.

Discussion

Least cost ration formulation programs based on linear modeling principals have existed for several years. However, these models were based on minimizing cost/cow, and do not reflect potential interactions with other activities and constraints of the farm system. To date few models have been developed to analyze either dietary effects alone or in combination with other management techniques on reproductive performance of dairy cattle, and how those effects may impact farm income (McCullough and DeLorenzo, 1996). Refining the model to include such considerations as specific fatty acid and amino acid supplementation to improve animal fertility and nitrogen efficiency will allow dairyman to analyze the economic value of these nutritional factors. Balancing animal requirements with effluent uptake by feed crops using the linear model approach can assist the dairyman by predicting optimal use of land and feedstuffs (Henry et al., 1995).

Additions to the model were made to better weigh the benefits and costs of supplemental dietary fat and low-dose bST for improved reproductive efficiency. As more data become available, the model may be refined to better reflect the expected benefits of these treatments. In order to thoroughly examine the effects of nutrition and hormonal treatments on calving interval and long term reproductive efficiency and milk production, as well as the possible benefits of raising on-farm replacement heifers on genetic advancement, a multi-year dynamic model would need to be constructed. Due to limitations of the Quattro Pro spreadsheet program, another software system would have to be employed to accommodate a larger model.

Considerable interest has been expressed by dairymen in the southeast in intensive grazing as an alternative to confinement housing and use of TMR. Intensive grazing may be introduced into the model to compare with the current confinement management system. Such an option would necessarily include effects of grazing in the south on milk production and fertility, as well as predicting an optimal supplemental diet for animals in such a system.

Linear modeling is a useful and highly flexible tool for analyzing decision making and predicting the value of certain options. However, the current method for modeling requires considerable training in order to produce, understand and interpret results. In order to use the model to assist individual dairymen, a more user-friendly format could be developed in which the user could enter constraints applicable to his/her particular farm situation.

Table 6-1 Comparison of N vs. N+P balance, maximizing income or minimizing costs.

	Nitrogen balance				Phosphorus Balance			
	Maximize Income Summer	Maximize Income Winter	Minimize Cost Summer	Minimize Cost Winter	Maximize Income Summer	Maximize Income Winter	Minimize Cost Summer	Minimize Cost Winter
Land Use (ha)								
pasture	288.31	288.31	219.50	298.19	240.02	240.02	230.24	85.37
crops	357.99	562.66	310.78	422.22	406.28	563.40	416.06	560.93
barns(m^2)	37,845.00	37,845.00	36,331.20	37,845.00	37,845.00	37,845.00	36,331.20	37,845.00
parlor (man/d)	28,509.90	28,509.90	27,219.36	28,509.90	28,509.90	28,509.90	27,219.36	28,509.90
high herd (h)	414.00	562.00	379.00	562.00	414.00	562.00	395.00	562.00
med herd (h)	1,522.00	1,526.00	1,393.00	1,526.00	1,522.00	1,526.00	1,453.00	1,526.00
low herd (h)	587.00	435.00	537.00	435.00	587.00	435.00	561.00	435.00
dry herd (h)	1,019.00	393.00	479.00	360.00	523.00	329.00	500.00	329.00
heifers (h)		0.00		688.00		0.00		793.00
yearlings (h)		830.00		688.00		840.00		793.00
Sales(h)								
vealers		1,340.00		1,111.00		842.00		828.00
heifers		1,340.00		423.00		842.00		828.00
culls		712.00		590.00		738.00		743.00

Table 6-1(contd.) Comparison of N vs. P balance, maximizing income or minimizing costs.

	Nitrogen balance		Phosphorus Balance	
	Maximize Income	Minimize Cost	Maximize Income	Minimize Cost
Crops (MT)				
silage	1,270.30	3,960 17	1,270 30	5,392.10
cereal hay	3,858 71	2,962 61	1,290 83	3 862.78
bermudagrass	1,303.10	3,710 38	2,000 53	1,206.15
Costs ($)				
bST	131,196.00	128,226 60	131,196.00	131,196 00
TAI	40,238.10	3,859 53	5,226.40	3,557.23
Labor(FTEs)	32.14	30 04	32.11	30 96
Milk (MT)	22,621 37	22,000 00	22,621 37	22,000 00
cost/c/d($)	2.97	1 91	3 58	2 09
cost/MT milk($)	159 58	91-96	172.06	100 76
Gross Margin($)	7,070,698 00	5,007 431.00	6,279,981 00	4,490,304 00

Table 6-2.Comparing technologies for maximum income under N budget.

	-TAI, -bST		-TAI, +bST		+TAI, -bST		+TAI, +bST	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Land Use (ha)								
pasture	303 98	303 98	330 73	330 73	307 16	307 16	288.31	288.31
crops	342 32	562.94	315 57	562.94	339.13	562 66	357 99	562 66
barns (m^2)	37,845 00	37,845 00	37,845 00	37,845 00	37,845 00	37,845 00	37,845 00	37,845 00
parlor (min/d)	28,509 90	28,509 90	28,509 90	28,509 90	28,509 90	28,509 90	28,509 90	28,509 90
Animals (h)								
high herd	414 00	562.00	414 00	562.00	414 00	562 00	414 00	562.00
medium herd	1,522 00	1,526 00	1,522 00	1,526 00	1,522 00	1,526.00	1,522 00	1 526 00
low herd	587 00	435 00	587 00	435 00	587 00	435 00	587 00	435 00
dry herd	1,013 00	329 00	523 00	329 00	1,173 00	393 00	1,019 00	393 00
yearlings		995 00		1,054 00		867 00		830 00
Annual sales (h)								
vealers		1,045 00		1,007 00		1,289 00		1,340 00
heifers		1,045 00		1,007 00		1,289 00		1,340 00
culls		877 00		929 00		742 00		712 00

Table 6-2(contd.) Comparing technologies for maximum income under N budget.

	-TAI, -bST	-TAI, +bST	+TAI, -bST	+TAI, +bST
Crops (MT)				
silage	1,730.32	1,232.42	1,774.80	1,270.30
cereal hay	3,860.65	3,759.34	3,858.71	3,858.71
bermudagrass hay	310.98	0.00	120.48	1,303.10
bST cost ($)	0.00	131,196.00	0.00	131,196.00
TAI cost ($)	0.00	0.00	40,238.06	40,238.10
Labor (FTEs)	44.21	31.62	44.16	32.14
Milk Production(MT)	19,670.75	22,621.37	19,670.75	22,621.37
cost/cow/day ($)	1.77	3.23	2.91	2.97
cost/MT ($)	193.76	179.66	185.76	159.58
Gross Margin ($)	5,542,494.00	6,288,850.00	5,930,196.00	7,070,698.00

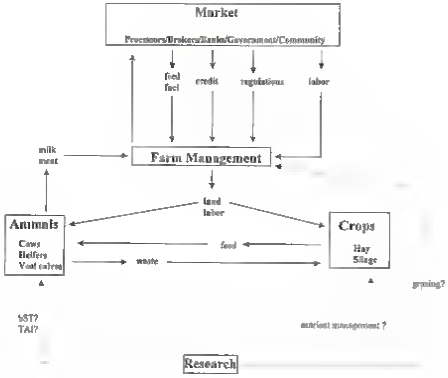


Figure 6-1 Schematic model of interactions between the farm enterprise, market and research.

CHAPTER 7
SUMMARY AND CONCLUSIONS

The dairy industry faces many challenges, not least of which is maintaining profits in the face of increasing input costs, decreasing prices for outputs, and changing government policies. It is the responsibility of scientists, extensionists, and consultants to provide dairy producers with as much information as possible, in a variety of formats, to help producers make decisions to better insure the continued health of their businesses. Meeting the nutritional requirements of animals in various stages of production is only part of the equation. However, as feed costs represent the largest share of expenditures, it is certainly among the most important considerations. In addition, reproductive efficiency has become a major issue of concern, as the productivity of lactating cows is greatest shortly after calving, and declines as lactation advances. Environmental regulations on dairies in Florida have introduced additional challenges to recycle manure nutrients to prevent increased N and P concentrations in ground water supplies.

In the current research, dietary fat was studied to examine its impact on rumen fermentation and the changes that occur in PUFA as a result of microbial hydrogenation *in vitro*, as well as the effects of a commonly used source of supplemental fat and protein (WCS) on animal health, production and reproduction. In the *in vitro* study, fiber digestibility, as measured by NDF disappearance, was unaffected by fat source or method

of incorporation into the diet. This confirms earlier findings from whole animal studies which measured total tract NDF digestibility (Drackley et al., 1994) and suggests that physical coating of fiber is unlikely to be the reason for reduced fiber digestion commonly observed in whole animal trials. High levels of alfalfa hay in the media (>30% of DM) may have prevented negative effects of PUFA on activity of rumen cellulolytic bacteria, possibly through the release of calcium and subsequent formation of insoluble soaps. Further investigations *in vitro* using corn silage alone or other types of forage low in calcium may be conducted to better elucidate the dynamics of interactions among sources of dietary fat and fiber, and rumen bacteria.

Biohydrogenation of PUFA, particularly of 18 carbon fatty acids, followed similar patterns regardless of fat source or method of incorporation. This was true of Ca-LCFA of PF, although extent of hydrogenation of 18:2n6 was less in calcium soaps compared to untreated poultry fat. Sukhija and Palmquist (1990) had noted that stability of high PUFA soaps was less than that of more saturated fatty acid soaps and that the pKd of high PUFA soaps was lower than that of the more saturated soaps. The buffer used in these experiments maintained pH at levels >6.0, which would likely have prevented significant amounts of dissociation of fatty acids. Also, the process of soap manufacture resulted in relatively large particle sizes, which would have limited the surface area available for bacterial attachment, and this may have prevented enzymatic hydrolysis of glyceride bonds.

Whole cottonseed had no effect on milk yield or composition in this study. Bovine somatotropin increased milk yield compared to no bST treatment. Milk response to WCS

has varied considerably among trials (Smith and Harris 1992, Staples et al , 1991) Fat percentage appears to be increased when alfalfa is included in the diet (Smith et al , 1993) A previous investigation had indicated that alfalfa hay content of the diet may be important in maintaining milk fat production, and that less than 12% of the DM may not be adequate to prevent milk fat depression (Adams et al., 1955) However, rations in the current experiment contained only 9 to 11 % alfalfa. Effects of dietary fat on protein percentage have also varied, but the mechanisms for milk protein depression have still not been elucidated

Whole cottonseed contains >50% 18 2ω6 fatty acids. This fatty acid has been shown to inhibit $PGF_{2\alpha}$ synthesis by uterine epithelium *in vitro* (Danet Deneyers, et al , 1993). To determine if this effect occurs *in vivo* two other pieces of information are required an estimation of escape of 18 2ω6 from hydrogenation in the rumen, and subsequent metabolic fate of 18 2ω6 Sampling of duodenal digesta was not possible in this experiment. Although percentage of 18.2ω6 as a proportion of total fatty acids in plasma TAG was not different between dietary treatments, quantity of TAG was increased in plasma of cows receiving WCS compared to those on no WCS diets While this would have caused an increase in total milligrams of 18 2ω6 in plasma TAG, that increase was only approximately 0 34 mg, which may not have been adequate to cause a difference in $PGF_{2\alpha}$ synthesis. Chromatography of plasma NEFA was not performed in the current investigation However, as *trans* 18 1 fatty acids were virtually undetectable in plasma TAG, but were increased in milk fat of WCS fed cows–suggests the possibility that fatty acids of dietary origin such as *trans* 18 1 and 18 2ω6 may also be contributed to the

mammary gland and peripheral tissues by plasma NEFA The extent to which PUFA accumulate in uterine epithelial tissue *in vivo* and thus may interfere with prostaglandin synthesis remains to be determined

Whole cottonseed did not affect pregnancy rates to TAI in the first two postpartum inseminations, even though plasma P_4 increased at a more rapid rate in cows receiving WCS compared to other treatments, and P_4 was higher in cows before injection of $PGF_{2\alpha}$ during first TAI Whole cottonseed did not alter populations of class 1 or class 3 ovarian follicles, but did increase the number of class 2 follicles in cows included in ultrasound observations Cows fed WCS also had larger subordinate follicles compared to cows on the no WCS diet.

Use of TAI in this trial was imposed in order to eliminate the variability in pregnancy rate due to reliance on heat detection, and was not to be tested as a treatment in the experiment However, it was noted that although cows receiving WCS had greater plasma P_4 concentrations prior to $PGF_{2\alpha}$ administration all animals returned to basal levels of P_4 (<1 0 ng/ml) by the time of insemination Timed AI may have eliminated any advantage conveyed by supplemental fat to reproductive responses in cows fed WCS

The whole animal study was also used to test the potential for use of a lower dose of bST as a therapeutic agent for stimulating reproductive activity in the early postpartum cow and thus increasing conception rates to TAI in the first 120 d of lactation. Exogenous bST is most widely used to stimulate milk production and increase the efficiency of conversion of feed to milk, thus enhancing animal productivity and

profitability However, reports regarding effects of bST on reproductive efficiency have been conflicting, and appear to depend on dose and physiological status of animals.

Exogenous bST injected at approximately half the recommended dose for commercial use, did not influence milk production or composition Bovine somatotropin appeared to enhance the dominance of preovulatory follicles in cows not consuming WCS when ovaries were monitored by ultrasound, resulting in fewer class 2 follicles and a smaller subordinate follicle

Although main effects of treatment on pregnancy to TAI was not significant, an interaction of parity x season of calving x bST was detected. Primiparous animals which calved in the warmer months at the beginning of the trial had a 40% greater pregnancy rate when receiving bST compared to similar animals which did not receive bST Somatotropin did not affect animals which calved in the cooler months, nor did it affect pregnancy rates among mature cows First parity animals suffering from heat stress tend to lose more body condition and take more time to recover than more mature animals managed under the same conditions (Thatcher, personal communication) These animals may represent a target group for use of low-dose bST to stimulate reproduction. Cows do not produce enough milk in their first lactation to return the dairyman's investment, and do not begin earning a profit until their second lactation (Bachman, personal communications) Poor reproductive efficiency extends the length of the first lactation when the cow represents a net loss Reproductive failure can lead to increased culling of primiparous cows, increase the need to purchase replacements and thus exacerbate the financial loss to the dairy producer

Interactions of WCS and bST were noted for BCS, plasma gossypol levels, EOF, and dominance of preovulatory follicles. Cows receiving both WCS and bST tended to have higher BCS scores at d30 postpartum compared to other treatments, and BCS tended to remain higher in this treatment group throughout the duration of the experiment.

Plasma total gossypol increased in a quadratic manner in cows fed WCS. In sharp contrast, cows consuming WCS and receiving bST experienced lower peak plasma gossypol levels, which declined sharply after 50 d postpartum. Gossypol is cleared from blood via the liver. It is possible that bST stimulated hepatic clearance of gossypol. Erythrocyte osmotic fragility was increased in cows consuming WCS alone, however, cows receiving WCS + bST had EOF values similar to that of controls. Increased EOF is a consistent finding in animals consuming cottonseed products high in gossypol (Risco et al., 1992, Gray et al.,1993). Although the biological significance of EOF is debatable, it may serve as an indication of how other cell membranes in the body may respond to gossypol concentration. The effects of bST on plasma gossypol has not previously been observed and warrants further investigation.

In the absence of WCS, cows treated with bST exhibited greater dominance of the preovulatory follicle, as evidenced by fewer class 2 follicles and a smaller subordinate follicle. In contrast, cows treated with both bST and WCS had more class 2 follicles and a larger subordinate follicle. It is unclear whether reduced dominance of the preovulatory follicle indicates development of a less viable oocyte. A less dominant follicle did not result in less functional CL in this trial, as cows receiving WCS had higher plasma P_4 levels than those which did not receive WCS.

Finally, a linear model was constructed using existing data to predict the impact of hormonal treatments and nutritional choices on whole farm dynamics, including nutrient balancing costs, production and gross marginal income. Unlike previous models, this program utilized data from an existing dairy and compared outputs to information provided by the dairy owner and manager to validate and correct inaccuracies in the programming.

Given the dynamics modeled on this large scale dairy (approximately 3000 adult cows) use of bST to increase milk production and feed efficiency and TAI to improve reproductive efficiency were selected as alternative technologies to increase gross marginal income of the farm. Timed AI was expected to increase profitability by reducing expenditures for replacement animals, however, culling and replacements were not reduced under TAI compared to a no TAI scenario. The benefit of TAI was through increased numbers of animals calving and entering the milking herd during the summer months, which resulted in increased annual milk output, and gross margin.

Profitability of the farm enterprise was reduced when P balance was imposed as a constraint in addition to N balance. Imposition of the P balance constraint resulted in the lowest gross margin compared to all other scenarios. Production and utilization of crop land varied depending on operative nutrient balance constraint, as well as whether the objective function was to maximize income or minimize cost. However, all scenarios reflected a need for a shift in land from existing pasture to crop production to ensure farm nutrient balance.

Under income maximization, all heifers born on farm were sold and all replacements purchased. Webb (personal communications) had advised dairymen that purchasing replacements was more profitable than raising replacement animals. This changed when cost minimization was the objective function. Some heifers were maintained on farm under cost minimization, but also heifers were sold. The owner of the dairy upon which the model was based raised all replacements on farm, and sold a few. Tauer (1998) noted that dairy owners do not always follow income maximizing behavior. Genetic improvement of livestock is difficult to accomplish with purchased replacements, as dairymen have little information or control over the genetic background and potential of purchased animals. Genetic improvement of the herd is generally cited as the reason for dairymen who choose to raise replacements on farm. The model does not currently include the potential financial benefits of genetic improvement.

Linear programming is an effective means of simulating real-life situations and predicting outcomes to changes in existing conditions. Use of linear modeling programs may be used effectively by dairymen, scientists, extensionists and consultants as a tool to evaluate complex management and nutritional decisions. Due to the large size of Florida dairies, a large scale operation was selected as the basis of the model. In order to assess the flexibility of the program, and its value to smaller dairy farms, additional data are needed from smaller scale operations.

APPENDIX
OUTPUT OF STATISTICAL ANALYSIS OF *IN VITRO* BIOHYDROGENATION STUDY

The SAS System 12 28 M [illegible] My/4 1988 1

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	20	0 1 1 3 4 5 6 7 8 9 10 11 11 11 18 15 16 17 18 19

Number of observations in data set = 113

The SAS System 12 11 Monday May 8 1998 1

General Linear Models Procedure

Dependent Variable: C11

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	89	18.86922208	0.10120710	1.92	0.0001
Error	288	2.70001011	0.02193981		
Corrected Total	112	28.03823229			

R Square	C.V.	Root MSE	C11 Mean
0.218701	60.00110	0.2682112	0.1346528

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	19	0.10112101	0.19038862	1.07	0.0001
TIME	1	3.22029021	2.71810011	11.86	0.0001
TIME*TRT	18	0.88111010	0.19719119	3.71	0.0001
TIME2	1	0.01102580	0.05281111	0.10	0.5001
TIME2*TRT	18	2.01005001	0.13708781	1.82	0.0191

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	19	1.31929101	0.13812126	8.65	0.0001
TIME	1	0.11902031	0.11012011	1.68	0.2007
TIME*TRT	18	1.10681011	0.13818875	3.18	0.0001
TIME2	1	0.02287881	0.02208855	0.15	0.1881
TIME2*TRT	18	1.11991001	0.12788288	1.92	0.0181

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	18	0.11920301	0.32812128	8.58	0.0001
TIME	1	0.02881221	0.07806211	1.61	0.2318
TIME*TRT	18	1.48581118	0.11018375	2.29	0.0001
TIME2	1	0.01210882	0.01216052	0.59	0.4858
TIME2*TRT	19	1.61981001	0.11788788	1.92	0.0191

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	18	9.11920500	0.33112331	8.65	0.0001
TIME	1	0.07886221	0.07886211	1.61	0.3118
TIME*TRT	19	1.10101125	0.11011928	3.28	0.0001
TIME2	1	0.08210882	0.04211052	0.18	0.1886
TIME2*TRT	18	2.01000001	0.11788788	1.92	0.0188

Dependent Variable: C15 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	88	38 68248565	0 88258351	17 30	0 0001
Error	888	4 19751838	0 13855682		
Corrected Total	107	88 88888184			

R-Square	C.V.	Root MSE	C15 1 Mean
0 884338	58 08837	0 1078867	0 8488088

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	88	87 84388326	1 46823438	37 57	0 0008
TIME	1	2 53576878	8 53878881	60 97	0 0001
TIME*TRT	88	5 58858881	0 28315543	7 88	0 0008
TIME2	1	0 35129785	0 35135815	8 47	0 0138
TIME2*TRT	88	3 58153513	0 13587887	8 58	0 0008

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	88	8 32685288	0 05879437	8 80	0 0221
TIME	1	0 98885868	0 88888488	35 45	0 0001
TIME*TRT	88	8 81726143	0 24388275	8 88	0 0001
TIME2	1	0 35129788	0 35138815	8 87	0 0138
TIME2*TRT	18	2 58153513	0 13587827	3 88	0 0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	89	1 32880388	0 05878437	8 88	0 0321
TIME	1	0 81385698	0 81356688	20 93	0 0001
TIME*TRT	18	8 48838142	0 38383388	8 28	0 0001
TIME2	1	0 18388878	0 18384185	4 73	0 0318
TIME2*TRT	89	8 88153513	0 12887888	3 58	0 0001

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	18	8 32685298	0 05878437	8 80	0 0321
TIME	8	0 81358588	0 81356058	38 03	0 0008
TIME*TRT	18	8 61838162	0 38388375	0 25	0 0001
TIME2	8	0 10398178	0 18384188	8 83	0 0388
TIME2*TRT	18	2 88853513	0 88588027	3 88	0 0001

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	1 11860871	0 01860110	1 01	0 0158
Error	188	3 58188388	0 03588892		
Corrected Total	181	7 58169888			

R-Square	C.V.	Root MSE	Y1 1 Mean
0 469115	371 5887	0 1828888	0 3119478

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	10	1 18858183	0 07877294	1 02	0 0181
TIME	1	0 53721888	0 81121888	1 01	0 3113
TIME*TRT	18	0 38858838	0 81811178	0 55	0 8110
TIME1	1	0 81187129	0 81187110	0 97	0 3251
TIME2*TRT	10	1 18885888	0 88885209	2 88	0 8021

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	10	0 80188811	0 88818569	1 28	0 2082
TIME	1	0 75180471	0 81181811	1.58	0 2230
TIME*TRT	10	1 57825588	0 88838187	1 40	0 8021
TIME2	1	0 81187129	0 81187110	0 87	0.1151
TIME1*TRT	10	1 18188868	0 88885109	2 88	0 8021

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	10	0 80188811	0 88818569	1 28	0.2082
TIME	1	0 80120871	0 80110871	1 11	0 1141
TIME*TRT	10	1 67811188	0 88818181	2.88	0.0015
TIME2	1	0 87853138	0 87881138	1 81	0 1178
TIME2*TRT	10	1 18868868	0 88881109	2 88	0 8021

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	10	0 80188811	0 88818569	1 28	0 2082
TIME	1	0 81328813	0 81320871	2 11	0 1188
TIME*TRT	10	1 67821188	0 88818181	2.88	0 0015
TIME2	1	0 81883138	0 81881138	1 81	0 1178
TIME2*TRT	10	1 78888868	0 88881109	1 88	0 8021

Dependent Variable C18

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	53	3865.8826438	85.8880160	11.	0.0001
Error	103	690.7723883	3.3627062		
Corrected Total	133	8038.8383134			

R Square	C V	Root MSE	C18 Mean
0.885727	26.58868	2.3585398	8.8811858

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	10	558.8737581	88.6565138	3.30	0.0001
TIME	1	3380.1838330	3380.1338330	380.38	0.0001
TIME*TRT	10	837.8788688	33.3187310	3.33	0.0038
TIME2	1	129.0117303	133.0113303	22.38	0.0001
TIME2*TRT	10	153.5177997	8.2377308	1.38	0.1338

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	10	188.35283163	8.81114081	1.78	0.0338
TIME	1	7.33333338	3.88333338	1.38	0.3318
TIME*TRT	10	117.73884171	8.18685082	1.11	0.3838
TIME2	1	133.31177645	128.31177083	33.38	0.6001
TIME2*TRT	10	138.33778838	8.83777833	1.38	0.1083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	10	188.31205165	8.81116083	1.78	0.0380
TIME	1	8.78833330	8.38833336	0.88	0.3553
TIME*TRT	10	117.33884171	8.18833880	1.11	0.3038
TIME2	1	188.38331883	188.38333388	25.88	0.0001
TIME2*TRT	10	138.51778378	8.23777833	1.88	0.1088

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	10	188.31805185	9.31116061	1.38	0.0380
TIME	1	8.78832230	8.38872238	8.83	0.3058
TIME*TRT	10	113.33888171	6.18658083	1.11	0.3838
TIME2	1	138.58331888	188.38331888	25.83	0.0001
TIME3*TRT	13	133.51773978	8.23777683	1.88	0.1038

Dependent [illegible]

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	58	1122 3718810	101 7060118	11 00	0 0001
Error	108	920 6280971	8 8700883		
Corrected Total	167	7089 0000000			

R-Square	C V	Root MSE	C10 1 Mean
0 868581	10 08700	2 8201888	27 185300

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	11	1771 1081817	110 7807708	28 18	0 0001
TIME	1	1108 8905171	1101 1000171	101 18	0 0001
TIME*TRT	10	301 9941718	18 8887000	1 88	0 0113
TIME2	1	828 0971711	116 0071711	61 12	0 0001
TIME2*TRT	11	101 4251780	8 0013163	0 65	0 0163

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	10	1032 1628318	88 1181171	11 11	0 0001
TIME	1	1010 1111140	1011 3200180	118 18	0 0001
TIME*TRT	10	111 2220801	0 1658701	0 70	0 7127
TIME2	1	818 0071711	111 0071711	11 11	0 0001
TIME2*TRT	10	100 1185788	8 0013162	0 65	0 0161

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	10	1032 1628118	88 1880378	11 21	0 0001
TIME	1	103 1101160	101 1190180	118 63	0.0001
TIME*TRT	10	111 1110401	8 8853708	0 78	0 7527
TIME2	1	110 8116000	100 0185000	10 18	0.0001
TIME2*TRT	10	101 1288783	1 4811162	0 61	0 8101

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	10	1882 8811115	10 1111171	11 11	0.0001
TIME	1	881 1108180	961 8810800	118 83	0 0001
TIME*TRT	10	123 2220101	0 1013701	0 78	0 7127
TIME2	1	100 8110000	898 8285008	58 18	0 0001
TIME2*TRT	10	188 8288780	1 0013462	0 81	0 0161

Dependent Variable: C16_FT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	88	82.81255518	1.58051077	2.01	0.0001
Error	100	50.05515007	0.55754769		
Corrected Total	187	188.86768870			

R-Square	C.V.	Root MSE	C16_FT Mean
0.434100	145.0861	0.7331764	0.5010155

Source	DF	Type I SS	Mean Square	F Value	Pr > F
T87	10	25.54000112	1.55480332	2.48	0.0017
TIME	1	0.00086145	0.00086145	0.00	0.8707
TIME*TRT	15	48.52705458	2.58410580	0.75	0.0091
TIME2	1	0.84705832	0.84702851	11.00	0.0000
TIME2*T87	10	0.98078050	0.05639735	1.10	0.2887

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	10	17.10221050	0.00327470	1.68	0.0580
TIME	1	0.78588378	0.78588388	10.77	0.0014
TIME2*TRT	10	5.08851050	0.50884064	0.57	0.0175
TIME2	1	0.84707851	0.84702851	11.00	0.0000
TIME2*TRT	10	0.98078050	0.05639735	1.10	0.2887

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	10	17.10221050	0.00327470	1.68	0.0800
TIME	1	0.22108871	0.55106671	0.71	0.0025
TIME*TRT	15	0.00851050	0.00884084	0.57	0.0175
TIME2	1	0.50750355	0.50750355	10.55	0.0030
TIME2*TRT	15	0.05070550	0.03637733	1.10	0.2887

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	15	17.10221050	0.00327470	1.68	0.0500
TIME	1	0.22108871	0.22108671	0.71	0.0021
TIME*TRT	18	0.00851050	0.00844014	0.57	0.0178
TIME2	1	0.50750355	0.50750353	10.55	0.0010
TIME2*TRT	18	0.05070550	0.03057755	1.10	0.2887

Dependent Variable: C18 [illegible]

Source	DF	Sum of Squares	Mean Square	[illegible]	[illegible]
Model	59	199 10093610	3 38001888	0 [illegible]	[illegible]
Error	108	279 59060909	9 59999038		
Corrected Total	167	911 99199999			

R-Square	C V	Root MSE	C18 2T Mean
0 929103	818 8780	1 9103649	0 2913910

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	18	88 89420723	2 96601907	0 89	0 5838
TIME	1	9 98509199	8 10000128	9 87	0 0982
TIME*TRT	18	60 98502721	3 19996909	1 28	0 2328
TIME2	1	1 96509158	1 10000100	0 99	0 9683
TIME2*TRT	18	19 91899029	1 01609929	0 80	0 9879

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	18	120 11386600	8 99859990	9 88	0 0018
TIME	1	9 62799199	3 62118108	1 82	0 2893
TIME*TRT	10	11 19838888	1 99917999	0 11	0 1421
TIME2	1	1 99919190	1 39909100	0 93	0 9699
TIME2*TRT	10	19 11989812	1 01602993	0 90	0 0018

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	19	120 11989989	9 92889990	2 98	0 0018
TIME	1	9 19996999	9 10018889	1 63	0 2091
TIME*TRT	19	11 19810888	1 99917998	0 11	0 1991
TIME2	1	1 99960091	1 99788881	0 69	0 9219
TIME2*TRT	19	18 31969812	1 01609629	0 90	0 8979

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	19	190 11399609	6 92899990	9 98	0 0018
TIME	1	9 19936999	9 19816988	1 99	0 2091
TIME*TRT	19	31 19838850	1 85917390	0 11	0 1891
TIME2	1	1 98299097	1 96299081	0 99	0 9919
TIME2*TRT	19	19 91999832	1 01989999	0 90	0 9070

Dependent Variable: C18_[illegible]

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	58	14483 453815	245 211868	[illegible]	0 0001
Error	108	5823 551031	56 551585		
Corrected Total	167	18408 878816			

R Square	C V	Root MSE	C18_2 Mean
0 342111	21 51833	5 0258321	51 550555

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	18	8510 0750515	453 8861883	0 62	0 0001
TIME	1	5895 0811555	5695 0811555	100 62	0 0001
TIME*TRT	10	712 8153813	33 5210085	0 81	0 6055
TIME2	1	0 0108515	0 0108515	0 02	0 8853
TIME2*TRT	18	580 7826033	29 8184638	0 03	0 8725

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	18	5550 5068181	138 3683588	5 53	0 0001
TIME	1	555 3185555	555 5155555	7 84	0 1867
TIME*TRT	10	486 1053005	23 4041533	0 50	0 0550
TIME2	1	0 0108515	0 0108515	0 02	0 8863
TIME2*TRT	10	580 7056073	38 8186635	0 85	0 0526

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	18	5530 5008181	133 3103888	3 55	0 0001
TIME	1	515 5951515	516 5851515	6 80	0 0108
TIME*TRT	18	548 1987285	23 8848335	0 80	0 8556
TIME2	1	0 0011855	0 0011855	0 00	0 9881
TIME2*TRT	18	560 5628035	28 8176835	0 63	0 8356

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	18	5558 5068181	138 5883588	5 53	0 0001
TIME	1	315 5951515	518 5851515	6 58	0 0185
TIME*TRT	15	556 1865085	23 4080375	0 50	0 8558
TIME2	1	0 0011835	0 0011836	0 00	0 8940
TIME2*TRT	18	580 5826075	28 5330635	0 63	0 8328

Dependent [illegible]

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	58	138.85059878	2.18022870	3.88	0.0001
Error	108	58.37383788	0.54874080		
Corrected Total	187	188.63081670			

R Square	C.V.	Root MSE	C18_3 Mean
0.688746	35.45317	0.7414513	3.2913853

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	18	23.87282858	1.30087383	3.30	0.0060
TIME	1	88.55488873	88.88888972	183.81	0.0001
TIME*TRT	18	18.66538838	0.77081048	1.40	0.1410
TIME2	1	0.11745429	0.11788829	0.21	0.8878
TIME2*TRT	18	7.37838888	0.38138473	0.71	0.8050

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	18	33.47888173	1.33373883	3.35	0.0040
TIME	1	8.27383658	8.27383650	0.58	0.0035
TIME*TRT	18	8.10416204	0.88357805	0.88	0.1888
TIME2	1	0.01785438	0.01745438	0.01	0.8873
TIME2*TRT	18	7.37830883	0.38838873	0.71	0.8082

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	18	33.47888173	1.33573863	3.25	0.0048
TIME	1	8.20855138	5.20495134	8.47	0.0037
TIME*TRT	18	8.38416274	0.88337655	0.88	0.1888
TIME2	1	0.01138338	0.01138338	0.02	0.8858
TIME2*TRT	18	7.37830885	0.38838873	0.71	0.8053

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	18	33.47889172	1.23572262	2.25	0.0048
TIME	1	5.20495134	5.30495138	8.47	0.0037
TIME*TRT	18	8.18816208	0.40337885	0.80	0.6886
TIME2	1	0.01136338	0.01136326	0.02	0.8858
TIME2*TRT	18	7.37830888	0.38838873	0.71	0.8082

Dependent Variable: C20 5

Source	DF	Sum of Squares	Mean Square	[illegible]	Pr > F
Model	58	855.71051255	0.81082155	[illegible]	0.0001
Error	108	60.71780888	0.75278777		
Corrected Total	167	557.52023751			

R-Square	C.V.	Root MSE	C20_7 Mean
0.881877	87.52258	0.7558025	0.7570750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	18	887.28843858	21.52620582	51.58	0.0001
TIME	1	1.58015087	1.58818787	2.51	0.1157
TIME*TRT	18	21.72771738	1.12555075	2.82	0.0128
TIME2	1	5.78085928	0.77077088	0.72	0.0057
TIME2*TRT	18	27.78377787	1.28838728	2.21	0.0055

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	18	88.51577787	0.75877705	0.45	0.0001
TIME	1	5.26022591	5.26022001	11.31	0.0011
TIME*TRT	18	28.07188817	1.52708555	2.72	0.0005
TIME2	1	5.78085928	0.78085920	0.72	0.0053
TIME2*TRT	18	23.64135757	1.25520725	0.21	0.0055

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	18	90.81677786	0.75077755	0.55	0.0001
TIME	1	5.78957045	5.75757287	10.27	0.0018
TIME*TRT	18	28.87188817	1.52785564	2.72	0.0005
TIME2	1	5.00215672	0.80218573	0.78	0.0082
TIME2*TRT	18	23.78777787	1.28538725	2.21	0.0055

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	18	80.81676785	5.75077705	0.55	0.0001
TIME	1	5.75857085	7.76977887	10.28	0.0018
TIME*TRT	18	28.07188015	1.52700564	2.72	0.0005
TIME2	1	0.00215572	0.00315577	0.55	0.0052
TIME2*TRT	18	23.64335757	1.25828725	2.21	0.0177

Dependent Variable: C22 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	101 16882180	1 01111800	[illegible]	0 0001
Error	100	8 10111500	0 08111162		
Corrected Total	111	110 38180011			

R Square	C V	Root MSE	C11_0 Mean
0 928101	18 11119	0 1011111	0 8829130

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	11	100 13220011	1 18864000	18 81	0 0001
TIME	1	0 12301101	0 11101181	8 81	0 0118
TIME*TRT	11	1 11101371	0 11111111	1 11	0 0167
TIME2	1	0 11101110	0 01100154	11 81	0 0011
TIME2*TRT	11	1 81100611	0 10119000	1 81	0 0010

Source	DF	Type II SS	Mean Square	F Value	Pr > F
TRT	11	11 00110100	1 21110111	11 81	0 0001
TIME	1	1 22500111	1 22100133	11 08	0 0001
TIME*TRT	11	1 00004110	0 70011251	1 11	0 0011
TIME2	1	0 01500111	0 91581118	11 21	0 0011
TIME2*TRT	11	1 06700011	0 10201101	1 10	0 0010

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	11	11 10010150	1 11150011	10 91	0 0001
TIME	1	1 10111010	1 10111000	10 01	0 0003
TIME*TRT	10	3 00000150	0 10021110	2 11	0 0011
TIME2	1	0 11100511	0 12501561	11 11	0 0010
TIME2*TRT	10	3 01100013	0 10119000	1 10	0 0050

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
TRT	10	11 01000100	1 11110111	10 01	0 0001
TIME	1	1 10011090	1 10111000	11 01	0 0001
TIME*TRT	10	1 50000010	0 10021294	1 11	0 0011
TIME2	1	0 02501001	0 92100511	11 11	0 0010
TIME2*TRT	11	1 10310111	0 10101100	0 20	0 0010

The SAS System 11 20 Monday May 0 1110 11

General Linear Models Procedure
Least Squares Means

TRT	C11 LSMEAN	Std Err LSMEAN	Pr > |T| H0 LSMEAN=0
0	1 01120001	0 00110111	0 0001
1	0 31071111	0 06900011	0 0001
1	0 10000160	0 00113111	0 0022
1	0 10060011	0 05100120	0 0100
1	0 09710023	0 00113111	0 0020
1	0 01001100	0 01110010	0 0001
1	0 00101601	0 00950011	0 0003
1	0 11101310	0 01100110	0 0001
0	0 01101301	0 00111111	0 0011
1	0 06011110	0 10051066	0 0001
10	0 10011111	0 00100011	0 0017
11	0 01001111	0 00100110	0 0011
10	0 00030010	0 09017111	0 0011
11	0 00001111	0 01111111	0 0001
10	0 51111000	0 00000001	0 0001
11	0 51110111	0 09101111	0 0101
11	0 00010113	0 00641101	0 0001
11	0 11011001	0 09511111	0 0008
10	0 15010010	0 00511111	0 0000
19	0 13310111	0 00907111	0 0002

TRT	C15_1 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0
0	1.58522581	0.06586288	0.0001
1	0.20071015	0.05068815	0.0001
2	0.21680851	0.07074708	0.0028
3	0.72771877	0.07150071	0.0018
4	0.15400500	0.07124508	0.0055
5	0.35784348	0.07186145	0.0001
6	0.55087282	0.08584788	0.0002
7	0.70287738	0.08754555	0.0032
8	0.55500848	0.07728788	0.0001
9	0.75787788	0.07550000	0.0005
10	0.55017145	0.08586209	0.0005
11	0.77888838	0.07155651	0.0051
12	0.18554515	0.05078788	0.0008
13	0.56715571	0.07054785	0.0001
14	0.59085085	0.05585088	0.0021
15	0.18888851	0.07054505	0.0086
16	0.54154511	0.08755578	0.0005
17	0.25851815	0.07854788	0.0019
18	0.28745088	0.07658588	0.0088
19	0.18555885	0.08508288	0.0050

TRT	C17_1 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0
0	0.15863836	0.06415588	0.0475
1	0.02545158	0.06417708	0.5155
2	0.02871062	0.08848651	0.7772
3	0.05155088	0.08047727	0.8504
4	0.08078631	0.08848051	0.4771
5	0.56415810	0.07701857	0.0005
6	0.10857718	0.08415588	0.0755
7	0.57688855	0.08551882	0.0008
8	0.08188855	0.06848051	0.1885
9	0.78888558	0.05385088	0.0001
10	0.08885558	0.05417588	0.1350
11	0.07887548	0.06585877	0.5705
12	0.05755825	0.06648851	0.5800
13	0.02188288	0.06888651	0.7488
14	0.15155588	0.08417588	0.0132
15	0.18471885	0.08884851	0.1504
16	0.55755155	0.08192587	0.0001
17	0.07518755	0.08848851	0.5805
18	0.01601355	0.08848851	0.8185
19	0.08618869	0.08815288	0.3048

TRT	C18 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0
0	10.5151804	0.7870558	0.0001
1	8.5185285	0.5050328	0.0001
2	8.1150558	0.8408058	0.0001
3	7.5576747	2.0558529	0.0001
4	7.5450758	0.8408059	0.0001
5	11.9001681	0.0597088	0.0001
6	15.1140690	0.7875556	0.0001
7	17.1088788	0.8044560	0.0001
8	7.8221085	0.8408058	0.0001
9	5.5858271	0.8016081	0.0001
10	8.8760718	0.5055576	0.0001
11	8.0875515	0.8525758	0.0001
12	10.5868152	0.8884028	0.0001
13	18.7815515	0.8888828	0.0001
14	7.0470788	0.7858558	0.0001
15	5.7871551	0.8408058	0.0001
16	7.5788551	0.5605808	0.0001
17	8.5514705	0.8404058	0.0001
18	7.7881209	0.8808078	0.0001
19	7.8545555	0.7875556	0.0001

TRT	C37_1 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0
0	37.3333343	0.3703381	0.0001
3	33.3333813	0.8703383	0.0003
7	37.3383308	3.0433338	0.0003
3	33.8138508	1.8887383	0.0003
4	27.8333920	3.0133338	0.0001
5	33.3333311	1.0873033	0.0003
8	33.3333338	0.9388881	0.0001
7	32.0083887	0.9800013	0.0003
8	33.0333333	1.0177778	0.0003
9	31.4033984	3.3388833	0.0001
10	33.3839652	0.0788581	0.0001
11	38.7378843	3.0383343	0.0001
13	33.9387313	1.0177778	0.0003
13	29.7878848	3.0437338	0.0003
38	33.9384600	0.9788581	0.0001
18	23.1838898	3.0437333	0.0003
38	28.3882430	0.8817928	0.0001
17	28.6988717	3.0137238	0.0001
38	33.0838838	1.0877734	0.0001
18	32.8838388	0.0703383	0.0003

TRT	C38_3T LSMEAN	Std Err LSMEAN	Pr > |T| H0 LSMEAN=0
0	1.78818873	0.34493788	0.0003
3	0.30983338	0.21883786	0.1697
7	0.77777283	0.38334797	0.3038
3	0.38838338	0.33333883	0.3828
1	0.13880718	0.38134887	0.8333
5	0.47393888	0.38331838	0.3368
6	0.17983817	0.78483783	0.4808
7	0.33848388	0.38333393	0.1628
8	0.78881847	0.38134787	0.3733
9	0.38387373	0.38839232	0.1628
18	0.18178728	0.74163788	0.9338
33	0.88833848	0.33333331	0.3782
12	0.58882343	0.33334390	0.8383
33	0.88133883	0.36138787	0.8181
34	0.18379317	0.34482788	0.8104
15	0.38773825	0.28178787	0.1182
38	3.03833887	0.33833888	0.6883
13	0.11838787	0.78338787	0.9487
38	0.38833813	0.38334387	0.0883
18	0.38778187	0.78183788	0.1883

TRT	C38 8T LSMEAN	Std Err LSMEAN	Pr > |T| H0 LSMEAN=0
0	0.87338387	0.33384383	0.3334
3	0.82143388	0.31788281	0.8678
7	7.73373188	0.33849827	0.0813
3	0.83838335	0.87731778	0.9853
8	0.77386828	0.33848933	0.6838
3	0.82348888	0.88287937	0.8623
8	0.83338848	0.33384383	0.0623
7	0.89337887	0.54533983	0.0394
8	0.33138848	0.36848933	0.9318
8	0.18318882	0.83181818	0.7921
18	0.87182926	0.37384383	0.3388
11	0.43883833	0.33883338	0.8873
33	0.83331628	0.56518527	0.9287
17	0.17312788	0.38848927	0.8813
34	0.38873888	0.33381781	0.7308
33	0.31187762	0.38848933	0.8833
14	0.83883333	0.33321828	0.6558
33	0.88818831	0.88948837	0.8838
18	0.37887873	0.38948827	0.8825
38	0.83643333	0.87781383	0.8380

[illegible]	C18_2 LSMEAN	Std Err LSMEAN	Pr > |T| HO LSMEAN=0
[illegible]	8.1111411	1.1791618	0.0001
[illegible]	31.0971181	1.1181818	0.0001
[illegible]	28.8661418	1.1711181	0.0001
[illegible]	43.1181781	2.1119118	0.0001
[illegible]	41.3190817	7.1711494	0.0001
[illegible]	24.0114681	7.1818811	0.0001
[illegible]	95.7808381	7.1799818	0.0001
[illegible]	1.1191171	2.3811111	0.0001
8	73.4877868	7.1111194	0.0001
9	10.1119641	1.1081114	0.0001
10	77.1787818	1.1113818	0.0001
11	14.1811182	2.4116111	0.0001
12	77.8181477	7.1111111	0.0001
13	11.1645081	1.4111484	0.0001
14	77.4787787	2.1713818	0.0001
19	22.0111118	2.4111484	0.0001
18	23.7174888	1.1111111	0.0001
11	11.9111081	1.4711484	0.0001
18	17.7874887	1.1711114	0.0001
11	41.1571811	2.2793111	0.0001

TMY	C18 1 LSMEAN	Std Err LSMEAN	Pr > |T| HO LSMEAN=0
0	1.10331111	0.11770111	0.0001
1	1.11811718	0.74770115	0.0001
2	1.81111167	0.16418811	0.0001
3	1.01287111	0.11814778	0.0001
4	7.10276381	0.11418811	0.0001
5	1.81817188	0.71826413	0.0001
6	1.79284811	0.11770119	0.0001
7	1.01110811	0.11181588	0.0001
8	7.71811119	0.11111811	0.0001
9	1.66110111	0.78711818	0.0001
10	7.81487831	0.14770111	0.0001
11	1.69481690	0.16814111	0.0001
12	7.82148818	0.74415611	0.0001
13	1.81188181	0.16418611	0.0001
14	1.89088888	0.11170111	0.0001
15	1.18118181	0.71418111	0.0001
16	7.12143811	0.11181718	0.0001
17	1.11114113	0.26418113	0.0001
18	1.78847147	0.18111811	0.0001
19	1.81891824	0.24118118	0.0001

TMY	C18 1 LSMEAN	Std Err LSMEAN	Pr > |T| HO LSMEAN=0
0	0.06377818	0.19841111	0.0007
1	0.01011762	0.25648114	0.9665
2	-0.00700000	0.78717111	1.0000
3	0.01110421	0.21116812	0.9610
4	0.00000000	0.10717117	1.0000
5	0.01191111	0.27130811	0.9658
6	0.00700000	0.19811118	1.0000
7	0.01113211	0.71874134	0.1812
8	0.00000000	0.10711111	1.0000
9	1.01122168	0.78854878	0.0119
10	0.01180000	0.75041130	0.1181
11	0.97711117	0.11111411	0.9780
12	0.14806470	0.78317217	0.9017
13	0.17086940	0.10711111	0.0111
14	4.01762101	0.21040130	0.0001
15	4.11000000	6.76317217	0.0001
16	4.17101717	0.11171110	0.0001
17	0.01111971	0.76719117	0.9111
18	0.00101911	0.21711111	0.5870
19	0.64111118	0.25041130	0.0020

	C23_8 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0
	0.18083603	0.03332383	0.5948
	0.75842498	0.03334403	0.4834
	0.13388328	0.10183088	0.4343
	0.04887448	0.10318144	0.7814
	0.13838698	0.10184886	0.1404
	0.77584144	0.10483883	0.4883
4	0.13435803	0.09533303	0.1948
4	0.01408803	0.08432184	0.8834
8	0.11433113	0.10183088	0.3624
3	0.44384383	0.10388228	0.0001
10	0.13338288	0.09532283	0.1948
11	0.33841938	0.10488134	0.2438
12	0.07883893	0.10184888	0.3841
13	0.13813978	0.10184888	0.4181
14	4.43413888	0.09333383	0.0001
15	1.43438714	0.10187888	0.0001
16	3.18218873	0.09138488	0.0001
17	0.08337232	0.10184888	0.3383
18	0.03811833	0.10167088	0.3688
19	0.08818888	0.08842288	0.5233

Dependent Variable: C13

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
ent v fat	1	0.04331084	0.04331084	1.05	0.3033
hom v sough	1	0.03348873	0.07333838	11.13	0.0013
ca v sh	1	0.00113484	0.00114684	0.92	0.4383
veg v an	1	0.32484802	0.32394882	4.83	0.0040
fs v other	1	0.04444043	0.04444043	1.04	0.3013
soug v tsp	1	0.18448447	0.18838333	3.33	0.1348
t v p	1	1.38388933	1.48888852	18.32	0.0701
c3 x c4	1	0.48847832	0.43847832	0.93	0.3394
c2 x c5	1	0.03333411	0.03433411	0.52	0.4423
c3 x c8	1	0.40884458	0.30383338	4.25	0.0414
c2 x c8	1	3.03386634	3.33388844	38.58	0.0001
c4 x c4	1	0.01484482	0.01884462	0.38	0.8114
c3 x c8	1	0.02333813	0.07553813	0.38	0.3326
c4 x c8	1	0.00124884	0.00142833	0.03	0.8384
c3 x c8	1	0.00131888	0.00151893	0.03	0.8830
time lin	1	0.07486231	0.04486221	1.03	0.3133
timequad	1	0.04210833	0.04210852	0.88	0.4436
clst1	1	0.00140348	0.00148873	0.33	0.3381
clst2	1	0.03033403	0.03524484	0.34	0.4873
c2 x t1	1	0.18808844	0.19903843	2.44	0.0841
c2x t3	1	0.08349431	0.06348431	0.97	0.3338
c3 x t1	1	0.00001888	0.00001600	0.00	0.6848
c3x t2	1	0.00044113	0.00044115	0.01	0.3343
c4 x t1	1	0.00884444	0.00834424	0.13	0.4184
c4x t3	1	0.00795233	0.00088258	0.01	0.3128
c8 x t1	1	0.15843418	0.13033313	3.03	0.1812
c9x t4	1	0.00348831	0.05848831	0.03	0.3653
c11 xt1	1	0.14488484	0.13369284	2.41	0.1441
c11xt4	1	0.03488283	0.03438283	1.44	0.4311
c13xt1	1	0.02488848	0.04330833	0.38	0.8348
c13xt4	1	0.01380384	0.01334904	0.10	0.8419
c15xt1	1	0.00482881	0.00333881	0.04	0.8443
c13xt3	1	0.00304318	0.00084218	0.03	0.8663

Dependent Variable: 35_1

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
ovt v fat	1	0.00032033	0.00032033	9.81	0.0078
hou v rough	1	0.00043030	0.00042900	0.03	0.3288
ss v sh	1	0.00000083	0.00000085	8.08	0.8803
veg v an	1	0.30775073	0.10333033	3.03	0.1077
fs v other	1	0.03030203	0.01839783	8.58	0.3833
snap v tip	1	0.01119830	0.00330330	0.33	0.8828
t v p	1	0.00730305	0.00736318	0.18	0.8623
s2 x c4	1	0.00001830	0.00001330	0.12	0.7711
c2 x c5	1	0.00000833	0.00000815	8.88	0.8073
c2 x s8	1	0.00000028	0.00000033	0.00	0.3737
c3 x c8	1	0.00000330	0.00000038	8.88	0.8897
s7 x c4	1	0.07190878	0.07190970	3.03	0.3783
c7 x c5	1	0.00053380	0.00050880	8.02	0.8821
s3 x s8	1	0.00277782	0.00277303	0.33	0.7367
s7 x c8	1	0.00303303	0.00187388	0.08	0.9838
time lin	1	0.01718778	0.03333080	38.03	0.0003
timequad	1	0.38333370	0.38708178	8.73	0.0330
slxt3	1	0.07703220	0.07303333	3.03	0.3762
slxt2	1	0.07033033	0.07037818	7.01	0.1303
s3 x t3	1	0.07310773	0.02330773	0.37	0.3318
c2s t2	1	0.07373373	0.07778535	0.01	0.3383
s3 x t3	1	0.00007303	0.00007303	0.00	0.3608
s7s t2	1	0.00003330	0.00003338	0.08	0.8887
s3 x t3	1	0.00014208	0.00006203	3.03	0.3303
s3x t2	1	0.00330033	0.00330023	0.88	0.3883
s8 x t3	1	0.00088788	0.00088783	0.03	0.8037
c8x t2	1	0.00121033	0.00330033	0.03	0.8837
s33 xt3	1	0.00177738	0.00177788	0.03	0.8318
t33xt3	1	0.03100833	0.00300303	0.33	0.7308
s13xt3	1	0.15878389	0.08875598	1.81	0.3378
c13xt2	1	0.03080000	0.03033000	1.70	0.7787
s15xt3	1	0.00320800	0.00320800	0.33	0.7333
s15xt2	1	0.00730330	0.00730330	0.18	0.6618

Dependent Variable: 3_1

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
cnt v fat	1	0.00673028	0.00675026	0.1?	0.8483
bmm v cough	1	0.00033030	0.00033030	0.03	0.6723
cv v s8	1	0.04081703	0.13001303	1.11	0.2833
veg v an	1	0.10313344	0.10013300	4.33	0.0330
fo v othec	1	0.11000340	0.11000640	3.01	0.0808
snap v tip	1	0.03330034	0.03330010	1.45	0.2300
t v p	1	0.0330010	0.75030015	3.31	0.1010
c3 x c0	1	0.00360003	0.00200003	0.?4	0.3003
c3 x c5	1	0.00738121	0.00008121	0.03	0.8523
c3 x c3	1	0.03040723	0.02036737	0.44	0.4300
c3 x c8	1	0.00440737	0.00439333	0.13	0.3883
c3 x c4	1	0.00703313	0.00333313	0.28	0.6533
c3 x c5	1	0.00288393	0.00308368	0.03	0.8104
c3 x c6	1	0.30303300	0.36300200	3.13	0.0800
c3 x cf	1	0.00008933	0.00033838	0.03	0.8302
time lin	1	0.00330433	0.3320373	3.43	0.1130
timequad	1	0.03461130	0.03403130	3.03	0.1039
c1xt1	1	0.00300033	0.00025603	0.13	0.8803
c1xt3	1	0.00344303	0.00344508	0.10	0.3365
c3 x t1	1	0.00003338	0.00003373	0.00	0.3729
c2x t3	1	0.00130042	0.00130003	0.03	0.8267
c3 x t1	1	0.13043013	0.13003317	3.44	0.0308
c3x t3	1	0.13000357	0.12800233	3.33	0.1633
c3 x t1	1	0.00300003	0.00300033	0.05	0.7341
c4x t2	1	0.00043302	0.00033303	0.13	0.3000
c? x t1	1	0.00330033	0.00333037	0.00	0.7031
c8x t2	1	0.00130401	0.00100381	0.03	0.8203
c11 xt1	1	0.31330440	0.31024362	0.?2	0.0001
c11xt3	1	0.07333041	0.03331331	10.33	0.0013
c12xt1	1	0.04333003	0.04334003	1.38	0.2038
c13xt2	1	0.03004303	0.03003303	1.??	0.3109
c13xt1	1	0.16033700	0.16033700	3.36	0.0330
c14xt3	1	0.10330400	0.10730300	3.31	0.0818

Dependent Va[illegible]

Contrast	DF	Contrast SS	Mean Square	F [illegible]	Pr > F
cnt v fst	1	1.69070338	1.69070774	0.3[illegible]	0.5813
hom v rough	1	0.21027048	0.21027088	0.04	0.8470
cn v sh	1	0.03333633	0.03333333	0.16	0.0833
veg v an	1	33.85858858	73.85858858	17.12	0.0008
fr v nthoc	1	0.88170831	0.34120831	1.34	0.3100
soap v tip	1	0.78163121	0.38183121	0.85	0.3208
t v p	1	38.38836438	38.39438438	8.30	0.0113
c2 x c4	1	1.88786837	1.88788835	0.3[illegible]	0.5577
c2 x c3	1	3.03030338	3.03030338	[illegible] 33	0.8848
c2 x c8	1	1.07030378	1.07030378	[illegible] 10	0.8818
c3 x c8	1	0.03300388	0.02300388	[illegible]	0.9880
c1 x c4	1	0.00044600	0.00544600	[illegible]	0.0030
c3 x c3	1	0.70288787	0.70288387	0.18	0.7088
c1 x c8	1	0.70785078	0.36743078	0.80	0.8165
c3 x c8	1	0.03293138	0.03285858	0.01	0.8852
time lin	1	0.78832338	4.78833330	0.34	0.3530
timequa3	1	148.38331888	184.38331848	33.80	0.0001
c1xt1	1	0.56551487	0.56581887	1.54	0.3173
c1xt3	1	0.01104638	0.01184638	1.00	0.2083
c2 x t1	1	3.70878388	3.30830388	0.50	0.8435
c2x t3	1	0.77300888	0.33300340	1.38	0.3118
c3 x t1	1	1.81277890	1.81737850	0.33	0.5697
c3x t3	1	5.03333838	3.03333830	1.00	0.3088
c4 x t1	1	0.18601523	0.14601833	0.83	0.8718
c4x t3	1	3.03302331	5.03300731	1.07	0.3030
c9 x t1	1	1.88333818	1.44333818	0.38	0.6118
c9x t3	1	0.00042883	0.00043085	0.00	0.8030
c11 xt1	1	0.00409843	0.00466843	0.40	0.8369
c11xt3	1	0.38088311	0.34888311	0.05	0.8028
c13xt1	1	0.03540182	0.42540182	0.15	0.7080
c13xt2	1	1.11888817	1.11885812	0.28	0.6588
c15xt1	1	3.18303846	3.18303840	0.37	0.8518
c15xt3	1	1.13130533	1.17325525	0.31	0.6408

Dependent Variable: C10 1

Contrast	DF	Contrast SS	Mean Square	[illegible]	Pr > F
cnt v fac	1	18.72253288	18.22253040	1.22	0.1928
hom v rough	1	3.01430180	2.01630180	0.28	0.0300
nn v ah	1	25.38821286	22.38421380	0.83	0.0032
veg v an	1	440.02838040	440.07838080	22.28	0.0001
fw v othoc	1	888.82836298	888.82828394	83.38	0.0001
snap v tig	1	12.38262013	12.38381012	2.82	0.1525
t v p	1	10.88138268	18.88828348	1.33	0.2338
c2 x c4	1	25.88735154	35.88083154	3.83	0.0827
c3 x c2	1	35.40613271	35.47013321	4.18	0.0439
c2 x c8	1	0.00882158	0.00883158	0.01	0.9288
c2 x c8	1	1.10810236	1.16009236	0.13	0.2282
c3 x c6	1	8.21884047	9.21888882	1.13	0.3080
c2 x c5	1	0.10482224	0.10442334	0.01	0.8132
c3 x c6	1	8.08783330	8.08782322	0.08	0.3301
c3 x c8	1	3.00828800	3.00454680	0.35	0.2222
time lin	1	885.55081960	883.28801891	110.67	0.0001
timequad	1	498.02548003	488.02580063	58.18	0.0001
c1xt1	1	22.11883354	25.11883254	2.92	0.0603
c1xt2	1	25.33332828	22.33333880	3.00	0.0862
c2 x t1	1	2.91235101	5.01235101	0.88	0.4133
c2x t2	1	8.88888822	8.48880833	0.88	0.3222
c3 x t1	1	32.38219382	35.39310383	8.13	0.0442
c3x t2	1	12.98150852	12.98120453	3.00	0.1588
c4 x t1	1	1.88864231	1.00864331	0.32	0.2330
c4x t2	1	8.53303143	8.27282143	0.03	0.3080
c8 x t1	1	10.22388224	10.33288538	1.38	0.3251
c8x t2	1	5.19513328	2.19512534	0.60	0.8822
c11 xt1	1	0.16288320	0.08388320	0.01	0.9284
c11xt2	1	0.38103088	0.38103088	0.04	0.8218
c12xt1	1	5.81681313	5.01881312	0.69	0.4081
c13xt2	1	8.18309872	8.18308423	0.40	0.8888
c12xt1	1	2.28288348	2.34388380	0.32	0.5330
c13xt2	1	3.00411153	2.00411152	0.33	0.8268

Dependent Variable C10 ST

Contrast	DF	Contrast SS	Mean Square	F Value	PR > F
cnt v fat	5	0.22755925	0.52721925	0.62	0.5570
hum v sough	1	0.55388501	0.53544595	0.55	0.5224
as v ah	5	2.71835650	2.75005005	5.84	0.0560
veg v an	1	5.05550575	5.02150575	0.75	0.1100
fo v galar	5	1.00355757	5.90559757	2.54	0.0625
soag v tip	1	0.55580754	0.52250755	0.07	0.3262
t v p	5	0.00375219	0.00571210	0.01	0.5550
c2 x a8	1	0.01055500	0.00085505	0.10	0.8771
a2 x c5	5	0.01555755	0.05455725	1.71	0.5857
a5 x a6	1	0.55570751	0.56570755	5.05	0.3077
a2 x a8	5	0.50765295	0.56755505	1.06	0.3085
a5 x a8	1	0.55750353	0.55750555	5.05	0.3555
a3 x c5	1	0.05075700	0.00675700	0.05	0.7587
a5 x a5	1	0.50572075	0.50575075	0.57	0.8055
c3 x c8	1	0.02577550	0.02177300	0.05	0.0500
time 55s	1	5.55500071	5.55590075	0.75	0.0053
timequad	1	5.50730555	5.50735353	10.25	0.0050
a5xt5	5	0.05700600	0.05700030	0.05	0.8555
c1xt2	1	0.00250027	0.00286027	0.05	0.5557
c2 x t5	5	0.05554036	0.05554050	0.50	0.7505
c2x t2	1	0.05592556	0.02492456	0.05	0.0500
c3 x t5	5	0.05574550	0.05575550	1.76	0.3400
c3x t5	1	0.55005325	0.02003351	0.70	0.5705
c4 x t1	5	0.05070710	0.50570750	0.07	0.3520
c4x t2	1	0.55555560	0.21121100	0.35	0.5205
c8 x t1	5	0.00089275	0.01059275	0.02	0.0002
c9x t5	1	0.00550775	0.00546773	0.55	0.0500
c11 xt1	5	0.07112615	0.07555050	0.15	0.7187
a55xt2	1	0.00507575	0.02207172	0.05	0.0850
c13xt1	5	0.22577550	0.55570495	0.85	0.5185
a55xt5	1	0.50575001	0.10005005	0.20	0.6570
c15xt5	1	0.02657225	0.02097525	0.05	0.8505
a15xt2	1	0.00257075	0.00219070	0.00	0.0894

Dependent Variable: C10_3T

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
cxt v fxt	1	1.13555831	1.13588831	[illegible]	0.5058
hem v rough	1	17.83888131	12.51886131	[illegible]	0.0798
cs v ah	1	1.71356805	1.73388805	[illegible]	0.0116
veg v ax	1	10.17178888	10.17178888	[illegible]	0.0588
fs v other	1	0.01887352	0.01587353	0.01	0.0373
esop v tsp	1	0.02338353	0.07339353	0.01	0.9235
t v p	1	1.82385600	1.57385600	0.56	0.4560
c2 x c5	1	31.87557635	21.57887888	8.41	0.0055
c7 x c8	1	30.10828130	35.10828130	10.18	0.0023
c7 x c8	1	0.02551585	0.07581905	0.01	0.9155
c7 x c9	1	0.58733067	0.58733333	[illegible]	0.5375
c3 x c4	1	0.08254333	0.08288733	0.03	0.8576
c3 x c5	1	0.75185037	0.75105033	0.28	0.5888
c3 x c8	1	0.31888873	0.31850673	0.17	0.7357
c3 x c9	1	0.53652661	0.57642661	0.18	0.6218
time lin	1	8.16978988	8.16938958	1.83	0.3051
timequad	1	1.86285657	1.55358087	0.58	0.4214
c1xt1	1	0.87655388	0.82055350	0.17	0.6835
c1xt7	1	0.33365550	0.32345060	0.09	0.7888
c9 x t1	1	3.85601338	3.68881338	1.55	0.3350
c9x t3	1	1.78562539	1.78862520	0.70	0.8885
c3 x t1	1	0.51568818	0.88885816	0.30	0.6581
c9x t3	1	0.28640530	0.25580530	0.16	0.7871
c4 x t1	1	3.28782808	3.28782544	1.38	0.3616
c9x t7	1	1.55871155	1.55571180	0.61	0.5387
c9 x t1	1	5.81510835	6.81818878	3.51	0.1160
c9x t2	1	3.88854273	3.85556233	1.30	0.7787
c11 xt1	1	0.81158127	0.81159123	0.00	0.9555
c11xt2	1	0.00581331	0.00581331	0.00	0.5828
c13xt1	1	0.83388598	0.13388558	0.01	0.9188
c13xt2	1	0.81605968	0.81888988	0.81	0.5359
c15xt1	1	0.11888388	0.11465302	0.05	0.8326
c15xt2	1	0.08505703	0.05609703	0.02	0.6851

Dependent Variable C19_2

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
cat v fat	1	333.3383325	333.3382333	3.33	0.0703
hum v rough	1	83.3783298	88.3283288	1.03	0.3882
ch v sh	1	33.2303833	23.7303333	0.33	0.8323
veg v an	1	3078.9381821	2828.8891871	88.88	0.0001
fo v other	1	333.3338358	333.3338358	3.33	0.0333
soap v tip	1	83.2383888	82.2882880	0.91	0.3828
t v p	1	28.3888803	38.3868303	0.33	0.3333
c2 x c4	1	333.7033701	253.7021781	5.38	0.0222
c2 x c5	1	198.2388288	303.3388283	3.33	0.1293
c2 x c3	1	38.3383838	38.2198333	0.81	0.3233
c2 x c3	1	32.3333353	32.3333351	0.88	0.3723
c3 x c3	1	33.3383858	33.3383838	0.73	0.3833
c3 x c5	1	8.6888388	8.6888396	0.81	0.9033
c3 x c3	1	3.3032800	3.3037800	0.13	0.7178
c3 x c4	1	3.7283833	3.7282833	0.12	0.7233
time lin	1	333.8931838	318.8931518	6.88	0.0108
timequad	1	0.8833833	0.9833833	0.58	0.3858
c3xt1	1	27.8910001	22.8818881	0.89	0.8831
c1xt3	1	33.4883333	33.3883333	0.33	0.3332
c2 x t1	1	0.8163854	0.6183858	0.02	0.9988
c2x t3	1	32.7333583	37.7333383	0.68	0.3333
c3 x t1	1	28.1228818	29.1228988	0.60	0.8302
c3x t2	1	38.8277038	38.8220008	0.38	0.3362
c8 x t1	1	28.8208318	28.8388318	1.73	0.1311
c3x t3	1	303.8873233	303.8823733	3.38	0.3323
c9 x t1	1	23.8882298	23.8882288	0.80	0.8233
c9x t2	1	30.3382682	30.3382383	0.22	0.6363
c11 xt1	1	2.3538232	2.3835732	0.05	0.8223
c31xt3	1	3.3333868	3.3333858	0.03	0.8763
c33xt3	1	13.1283828	11.1288338	0.38	0.6288
c33xt2	1	30.3333833	10.3333828	0.33	0.3380
c35xt3	1	0.8832833	0.8832833	0.02	0.9802
c18xt2	1	3.9932888	3.9932033	0.08	0.7703

Dependent Variable: C18_5

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
org v fss	1	1.08822810	1.08822510	[illegible]	0.1018
hom v rough	1	0.32855555	0.32852550	[illegible]	0.0081
cs v ak	1	1.38588751	1.08880701	2 [illegible]	0.1155
veg v ss	1	1.82580501	1.55580501	2.7	0.0080
fs v other	1	0.88551557	0.88721858	1.40	0.5558
soap v tip	1	2.88588585	5.85558555	5.97	0.0570
s v p	1	0.11621827	0.11821558	0.21	0.8555
c2 x c5	1	0.88885518	0.88785815	1.51	0.5055
c2 x c5	1	0.85271828	0.86281888	0.88	0.8018
c2 x c5	1	0.18738557	0.10055558	0.55	0.5575
c2 x c5	1	0.78585468	0.78285858	1.88	0.2588
c3 x c8	1	2.02888181	5.05858881	5.15	0.0555
c3 x c5	1	1.55585581	1.28885281	2.28	0.1881
c3 x c6	1	0.67588888	0.67288758	1.55	0.5818
c5 x c6	1	0.51855828	0.51885828	0.88	0.8869
time lin	1	8.50455155	5.57555155	5.58	0.0857
timequad	1	0.01135556	0.01188888	0.02	0.8888
c1xs1	1	0.07788851	0.08778851	0.15	0.7075
c1xs2	1	0.05128282	0.08128285	0.18	0.8818
c2 x s1	1	0.57551555	0.58551592	0.58	0.5025
c2x s2	1	0.18881882	0.18881882	0.88	0.5888
c3 x s1	1	0.00155588	0.00155388	0.00	0.5855
c3x s5	1	0.56515868	0.56515858	0.15	0.7515
c4 x s1	1	0.05888555	0.05888555	0.05	0.0158
c4x s2	1	0.88828881	0.88828953	0.08	0.8151
c9 x s1	1	0.75892551	0.43885551	1.55	0.5515
c9x s2	1	0.58185885	0.88188978	1.05	0.3188
c11 xs1	1	0.55858815	0.22858515	0.50	0.5285
c11xs2	1	0.35888888	0.88848888	0.68	0.5284
c13xt1	1	1.55815550	1.55815550	5.07	0.1085
c13xt5	1	0.88181318	0.58181818	1.78	0.1888
c15xt1	1	0.55858558	0.15838555	0.05	0.7627
c15xs5	1	0.05586285	0.05968585	0.08	0.7885

Dependent Variable: C28_5

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
ant v fag	1	0.01715715	0.01754755	1.85	0.2305
ham v rough	1	0.15834558	0.15954555	0.58	0.5057
ec v Bb	1	1.33318508	1.30318508	1.88	0.1641
veg v an	1	8.33336553	8.55535587	14.01	0.0002
fs v other	1	72.75313308	33.36233318	138.02	[illegible]
sosp v Bip	1	0.00353368	0.00555380	0.01	0.3558
t v p	1	0.01335855	0.00203853	0.01	0.8035
c2 x c4	1	0.13503551	0.07503551	0.15	0.7181
c2 x c5	1	0.00701500	0.00075558	0.00	0.0008
c2 x c6	1	0.50615505	0.50015505	1.00	0.1058
c2 x c8	1	0.00550330	0.00500328	0.01	0.0133
c3 x c4	1	0.53033030	0.55453020	0.85	0.3518
c5 x c5	1	0.00355565	0.00025500	0.00	0.0833
c3 x c8	1	3.83002004	5.45005004	5.12	0.0150
c5 x c8	1	0.00008700	0.00004700	0.00	0.0001
time lin	1	5.35053045	5.75953045	10.55	0.0018
timequad	1	0.00318033	0.00314573	0.54	0.0082
clxt1	1	0.05170400	0.05140000	0.37	0.3033
clxt2	1	0.35033031	0.55053031	0.64	0.4553
c7 x sl	1	0.07605576	0.07605576	0.18	0.7138
c2x s5	1	0.23000030	0.22000030	0.60	0.5283
c5 x sl	1	0.41501504	0.41001204	1.45	0.5100
c3x t2	1	1.30003006	1.30007000	3.18	0.0303
c4 x sl	1	5.14150005	5.54150000	5.00	0.0158
c4x s3	1	3.00033050	2.00022650	5.00	0.0281
c0 x sl	1	0.04111515	0.04111215	0.03	0.3078
c9x t2	1	0.11333133	0.11313153	0.21	0.6408
c11 xt1	1	1.70555105	1.70555105	5.05	0.0087
c11x83	1	0.83041818	0.83041818	1.55	0.2181
c15x81	1	0.10718505	0.30718505	0.00	0.8005
c13xt2	1	0.01003543	0.01007543	1.44	0.5532
c15x81	1	0.37003858	0.33003858	0.57	0.4028
c15xt5	1	0.01054400	0.01054400	0.05	0.0555

Dependent Variable CH2 G

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
cgt v fsd	1	0.36800000	0.36860000	4.58	0.0338
hom v rough	1	0.03082033	0.03082033	0.38	0.5878
ca v ah	1	0.00320000	0.00320030	0.03	0.8611
veg v an	1	2.88805818	2.35808888	30.38	0.0001
fr v other	1	30.33733304	30.33735308	833.80	0.0003
soap v Bhp	1	0.08880058	0.08830000	0.53	0.3062
s v p	1	0.00300338	0.00300033	0.08	0.7783
c3 x c4	1	0.08888838	0.08738830	0.32	0.2538
c3 x c5	1	0.00333005	0.00333003	0.08	0.7908
c3 x c6	1	0.30328018	0.30035008	3.33	0.3580
c4 x c3	1	0.00300018	0.00080031	0.02	0.8803
c3 x c8	1	0.08880078	0.08808300	0.35	0.8835
c4 x c5	1	0.00330087	0.00530007	0.31	0.7332
c3 x c6	1	0.08882888	0.08342878	3.02	0.3388
c3 x c8	1	0.03352388	0.03823807	0.38	0.3358
Sims lin	1	4.88383000	3.33333808	38.[illegible]	0.0103
Simsquad	1	0.02583383	0.02586643	[illegible]	[illegible]
c3xt3	1	0.03380880	0.03333730	0.33	0.3765
c1xt2	1	0.03088333	0.08888832	0.63	0.8888
c3 x B8	1	0.03046383	0.03028338	0.38	0.3803
c2x t3	1	0.03002378	0.08882578	0.88	0.3858
c3 x B8	1	0.08027088	0.33088000	0.33	0.0333
c3x t2	1	0.87000033	0.87000685	3.88	0.0869
c3 x B3	1	0.08888883	0.03782308	0.38	0.0333
c8x t3	1	0.33000707	0.33000707	5.29	0.0233
c8 x B3	1	0.08881383	0.03800383	3.38	0.2833
c3x t2	1	0.05288333	0.00377885	3.33	0.3333
c33 xt3	1	0.00088328	0.03888883	0.88	0.7538
c88x88	1	0.00303548	0.00303838	0.08	0.8303
c33x83	1	0.08888088	0.03336238	0.83	0.3338
c88xt2	1	0.33323388	0.88322785	8.38	0.2808
c33x83	1	0.05381508	0.09300818	0.33	0.7333
c38xt2	1	0.33903253	0.08883258	1.88	0.2038

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BIOGRAPHICAL SKETCH

Amey L. Adams was born in Schenectady, N.Y. on July 26,1961 and lived much of her life on a small farm near Saratoga. Upon graduating with Honors from Niskayuna High School, she first attended the College of Saint Rose in Albany, N.Y., and later transferred to the State University at Albany, where she majored in English Literature with a concentration in Journalism.

After earning a Bachelor of Arts, Ms. Adams entered the Peace Corps and served three years in Honduras, C.A., as a small farm livestock extensionist. On completion of service she worked as a consultant in South Carolina and Honduras.

Currently the author is a candidate for the degree of Doctor of Philosophy with a major in Animal Nutrition. She plans to pursue a career in research and teaching.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

H H Van Horn
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Charles R. Staples
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

William W Thatcher
Graduate Research Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Peter E Hildebrand
Professor of Food and Resource Economics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Sean F O Keefe
Associate Professor of Food Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

December, 1998

Dean, College of Agriculture

Dean, Graduate School